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ERM European Reference Materials

CERTIFICATION REPORT

Certification of mass fractions of polychlorinated biphenyls (PCBs 28, 52, 74, 99, 101, 105, 110, 118, 138, 149, 153, 156, 177, 180, 183, 187, 194 and 196) in fish oil

Certified Reference Material ERM®-BB350

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Certification of mass fractions of polychlorinated biphenyls (PCBs 28, 52, 74, 99, 101, 105, 110, 118, 138, 149, 153, 156, 177, 180, 183, 187, 194 and 196) in fish oil

Certified Reference Material ERM®-BB350

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SUMMARY

This report describes the preparation of a salmon oil matrix certified reference material (ERM-BB350) and the certification of the content (mass fraction) of a selection of polychlorinated biphenyls (Nos. 28, 52, 74, 99, 101, 105, 110, 118, 138, 149, 153, 156, 177, 180, 183, 187, 194 and 196).

Certification of the CRM included testing of the homogeneity and stability of the material as well as the characterisation using an inter-comparison approach. The main purpose of the material is to assess method performance, i.e. for checking accuracy of analytical results. As any reference material, the CRM can also be used for control charts or validation studies.

Uncertainties were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [1] and include uncertainties due to possible heterogeneity, instability and characterisation. The certified values are listed below:

FISH OIL

IUPAC name (congener number) [1)]	Mass Fraction	
	Certified value [2)] [ng/g]	Uncertainty [3)] [ng/g]
2,4,4'-trichlorobiphenyl (PCB 28)	21.3	1.1
2,2',5,5'-tetrachlorobiphenyl (PCB 52)	37.4	2.2
2,4,4',5-tetrachlorobiphenyl (PCB 74)	23.0	1.9
2,2',4,4',5-pentachlorobiphenyl (PCB 99)	62	6
2,2',4,5,5'-pentachlorobiphenyl (PCB 101)	111	5
2,3,3',4,4'-pentachlorobiphenyl (PCB 105	25.8	2.1
2,3,3',4',6-pentachlorobiphenyl (PCB 110)	54.1	2.8
2,3',4,4',5-pentachlorobiphenyl (PCB 118)	84	4
2,2',3,4,4',5'-hexachlorobiphenyl (PCB 138)	137	10
2,2',3,4',5',6-hexachlorobiphenyl (PCB 149)	88	9
2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153)	220	11
2,3,3',4,4',5-hexachlorobiphenyl (PCB 156)	20.1	1.3
2,2',3,3',4,5',6'-heptachlorobiphenyl (PCB 177)	25.8	2.0
2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB 180)	67	4
2,2',3,4,4',5',6-heptachlorobiphenyl (PCB 183)	22.5	1.8
2,2',3,4',5,5',6-heptachlorobiphenyl (PCB 187)	67	5
2,2',3,3',4,4',5,5'-octachlorobiphenyl (PCB 194)	23.4	1.5
2,2',3,3',4,4',5,6'-octachlorobiphenyl (PCB 196)	41	7

1) As obtained by quantification using gas chromatographic methods. Numbering identical to that published by Ballschmiter K, Bacher R, Mennel A, Fischer R, Riehle U, Swerve M (1992) Journal of high-resolution chromatography 15, 206.

2) Unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory. The certified value and its uncertainty are traceable to the International System of Units (SI).

3) The certified uncertainty is the expanded uncertainty estimated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM) with a coverage factor $k = 2$ (with the exception of PCB 177 and 196, $k = 2.39$ and 2.66, respectively) corresponding to a level of confidence of about 95 %.

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Glossary

Δ_m	Absolute difference between mean measured value and the certified value
$v_{MSwithin}$	Degrees of freedom of MS_{within}
v_{smeas}	Degrees of freedom for the determination of the standard deviation s_{meas}
$\overline{y}$	Average of all results of a homogeneity study
ANOVA	Analysis of variance
BHT	Butylated hydroxy toluene
CRM	Certified Reference Material
ECD	Electron capture detection
ECNI	Electron capture negative ionisation
EI	Electron ionisation
GC-MS	Gas chromatography-mass spectrometry
HRMS	High-resolution mass spectrometry
IRMM	Institute for Reference Materials and Measurements
ISO	International Organization for Standardization
k	Coverage factor
LRMS	Low-resolution mass spectrometry
m/m	Mass/mass
MSD	Mass spectrometric detector
$MS_{between}$	Mean square between bottles from an ANOVA
MS_{within}	Mean square within a bottle from an ANOVA
n	Average number of replicates per bottle
No	Number
PCB	Polychlorinated biphenyl
POPs	Persistent Organic Pollutants
PTV	Programmable temperature vaporiser
RRF	Relative response factor
RSD	Relative standard deviation
SD	Standard deviation
se	Standard error
SI	International System of Units
s_{bb}	Between-bottle standard deviation
s_{meas}	Measurement variability
SPE	Solid phase extraction
s_{wb}	Within-bottle standard deviation
$t_{\alpha, df}$	Critical t-value for a t-test, with a level of confidence of 1-α and df degrees of freedom
u_{Δ}	Combined uncertainty of measurement result and certified value
u^*_{bb}	Uncertainty of inhomogeneity that could be hidden by method repeatability
u_{alt}	Alternative uncertainty
u_{CRM}	Standard combined uncertainty of a certified value
U_{CRM}	Expanded uncertainty of a certified value
u_{hom}	Uncertainty contribution of the homogeneity
u_{lts}	Uncertainty contribution of the long-term stability
u_m	measurement uncertainty
u_{sts}	Uncertainty contribution of the short-term stability

1 Introduction

Polychlorinated biphenyls (PCBs) are a class of 209 discrete chemical compounds in which 1 to 10 chlorine atoms are attached to a biphenyl backbone [2]. Commercial products of PCBs are complex mixtures that were widely used in industrial applications, such as transformers, capacitors, printing inks, paints, and paper industry. The use and/or production of PCBs has been banned in most developed countries since the 1970s [3]. Despite this measure, these compounds are among the most prevalent environmental pollutants. Their widespread presence is due to their extremely persistant and lipophilic nature. These properties cause these persistent organic pollutants (POPs) to bioaccumulate in the fatty tissues of biota, resulting in enrichment throughout the food chain [4]. PCBs can be found in nearly every environmental compartment, in human and animal fatty tissues, (human) milk, blood, sediment, sludge and marine and freshwater biota.

Prolonged exposure to these pollutants can interfere with normal physiology and biochemistry [5-8]. A wide range of toxicological and hormonal effects, including endocrine disruption, are induced by these environmental contaminants [9,10]. The occurence and severity of these interferences depend on various factors, such as the concentration of pollutants in the organism, susceptability of the species, and duration of exposure [11,12]. Several environmental and health agencies have already issued consumption advices, which range between 0.5 and 2 meals of fatty fish per month [13]. The general public faces seemingly conflicting reports on the risks and benefits of fish intake, resulting in controversy and confusion over the role of the consumption of fish and fish-derived products in a healthy diet [14].

Currently, PCB mass fractions in commercially available fish oils are relatively low compared to the oils that were available in the past. This can be explained by better sourcing of the fish used to produce the oil and by the improved clean-up processes. To be able to quantify all congeners of interest, the oil needed to be artificially fortified with PCBs. However, the relative abundance of the different congeners closely resembles the contamination profile that is found in fish.

From the 209 potential congeners, only about 120 have been detected in the environment. Since not all of these congeners can be analysed on a routine basis, a selection was made based on the following criteria: (i) presence in industrial mixtures, (ii) occurrence in environmental samples, (iii) toxicity, and (iv) analytical state-of-the-art. The following PCB congeners, numbered according to the scheme proposed by Ballschmiter et al. [15], were selected: 28, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 149, 153, 156, 163, 167, 170, 177, 180, 183, 187, 194 and 196. A full overview is given in Annex A.

The fish oil and its PCB pollution pattern is representative of the oil analysed in control laboratories and fisheries institutes. The PCB mass fractions are sufficiently high to quantify all congeners of interest and is suitable for the control and optimisation of analytical methods used to determine PCBs in lipophilic matrices.

2 Participants

2.1 Project management and evaluation

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34; BELAC No 268-TEST)

2.2 Processing

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(acredited to ISO Guide 34 n; BELAC No 268-TEST)

2.3 Homogeneity and stability studies

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Institute for Marine Research, Bergen, NO
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Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Institute of Ecological Chemistry, Neuherberg, DE
(accredited to ISO/IEC 17025 for measurement of PCBs using isotope dilution technique; DACH No DAC-PL-0141-01-10)

3 Material selection and processing

3.1 Material selection

It was not possible to find fish oil on the market that is contaminated with all PCBs of interest at such concentration that it would be a useful material as a basis for a certified reference material. Due to proper sourcing of the starting material for fish oil production and due to technological advances in clean-up procedures that are applied to the oil, the mass fractions of most PCBs are very low and it would have not been possible to certify those with an appropriate uncertainty. Therefore it was decided to fortify the material.
For the production of this reference material, salmon oil (*Salmo salar*) was obtained from a commercial fish oil supplier (United Fish Industries, Aberdeen, UK). The oil was prepared from salmon fish trimmings comprising of head, frames and viscera. The fish oil producer stabilised the oil by addition of 0.05 % mass fraction butylated hydroxy toluene BHT (0.5 mg/g).

3.2 Fish oil processing

As mentioned above, the salmon oil as provided by the commercial supplier had too low PCB content to facilitate certification of all congeners of interest. Fortification of the material was therefore necessary.
A solution containing all PCBs of interest was prepared in *iso*-octane. Around 45 g of this solution was added to 200 g of fish oil. The mixture was then heated to 45 °C and stirred using a magnetic stir-bar while being blown down under a constant N_2-flow until constant weight was obtained. The fortified oil was then further consecutively diluted with unfortified fish oil under constant stirring at 45 °C to a total mass of 6.4 kg. After each dilution step, the mixture was stirred at least 2 h before the next dilution was carried out.

After completion of all dilution steps, the nominal mass fractions of all PCB congeners of interest varied between roughly 20 and 200 ng/g oil. The final certified value represents the sum of the naturally present and the added amount of PCBs.

The fortified oil was stirred for 48 h at 45 °C before being ampouled. The ampoules were flushed with argon and filled with the fish oil that was kept at 45 °C during filling to ensure homogeneity of the mixture. Care was taken to prevent the oil from contaminating the neck of the vial, which was flame-sealed under argon immediately after filling. About 2000 ampoules of the salmon oil were prepared (around 2 g oil per ampoule). Care was taken to avoid contamination during all steps of the preparation and handling of the material.

3.3 Additional characterisation measurements

Water content of base material and filled ampoules were determined by vaporisation Karl Fisher titration [16]. From the base material, aliquots were taken and mixed (80 mL in total). This sample was analysed in triplicate. The determined mean water content of the bulk fish oil was 0.12 ± 0.01 g/100 g.
From the final material, 5 vials were chosen following a random stratified sample picking scheme and analysed in duplicate. The determined mean water content of the final material was 0.09 ± 0.01 g/100 g.

4 Assessment of homogeneity

Key requirement for any reference material is equivalence between the various units. In this respect, it is not relevant whether the variation between units is significant compared to the analytical variation, but whether this variation is significant to the certified uncertainty. Consequently, ISO Guide 34 requires RM producers to quantify the between bottle variation. This aspect is covered in between-unit homogeneity studies.
Within-unit heterogeneity does not influence the uncertainty of the certified value when the minimum sample intake is respected, but determines the minimum size of a subsample that is representative for the whole unit. Quantification of within-unit heterogeneity is therefore necessary to determine the minimum sample intake.

4.1 Between-bottle homogeneity

Between-bottle homogeneity was tested on 15 samples. These samples were taken using a random-stratified sampling scheme, thus ensuring that the complete batch was covered. As no influence was found for any of the conditions tested for the short-term stability assessment (Section "5.1 Stability during transport"), the fourteen samples that were used for the assessment of the short-term stability were used to assess homogeneity. In addition, one other sample was taken to come to a total of 15 samples.
Samples were measured in a random order to allow distinction between an analytical trend and a trend in the filling sequence. In order to exclude the influence of the day-to-day variance, all sample preparations were performed on the same day. Three replicate determinations per bottle were performed under repeatability conditions by means of a validated analytical method comprising SPE and GC/EI-MS. Sample intake was 70 mg. Details of the method used for homogeneity testing are given in Annex B. The homogeneity was assessed for all congeners that are certified.

4.1.1 Descriptive evaluation of between-bottle homogeneity of ERM-BB350

The individual data and bottle averages are first tested whether they follow a normal, or at least unimodal distribution, which was done by visual inspection of normal probability plots and histograms, respectively (consistently, 5 bins were chosen per histogram). If the data do not follow at least a unimodal distribution, the calculation of standard deviations can be doubtful or impossible. Grubbs-tests on 99 % confidence levels were performed to detect

potentially outlying individual results as well as outlying bottle averages. Regression analyses were performed to evaluate potential trends in the analytical sequence as well as trends in the filling sequence. The graphical representations of the results are depicted in Annex C. A summary of these evaluations are shown in Table 1.

A statistically significant trend in the filling sequence was observed for PCBs 101, 128, 170 and 196. Trends were also apparent in the analytical sequence for some congeners. However, as the analytical sequence was randomised and not correlated with the filling sequence, adjustments could be applied in the associated uncertainty were made to allow for these trends (Section "4.1.2 Estimation of uncertainty of homogeneity of ERM-BB350").

Some outlying results for individual analyses and bottle means were observed. The outlying results could not be attributed to storage conditions since all but one of the outlying values were recorded for samples stored at 18 °C and both high as low outliers were observed. A detailed overview of all outliers is given in Table 2.

No technical reasons for exclusion of outliers could be given. Therefore, entire data sets were kept to calculate the respective uncertainty related to the between-bottle variation (u_{hom}). The presence of the outliers was however taken into account for the assessment of the uncertainty of the homogeneity.

Table 1. Descriptive evaluation of the results for the homogeneity assessment of all individual PCB congeners in BB350. Data from the short-term stability study were used. If a significant slope (trend) could be observed in the data, the level of significance is given.

	Trends		Outliers		Distribution	
PCB	**Analytical sequence**	**Filling sequence**	**Individual results**	**Bottle means**	**Individual results**	**Bottle means**
28	95 %	no	none	one	normal	unimodal
52	no	no	one	one	unimodal	unimodal
74	no	no	none	one	unimodal	unimodal
95	no	no	two	none	unimodal	normal
99	95 %	no	none	none	normal	normal
101	99 %	95 %	none	none	normal	unimodal
105	no	no	none	none	normal	unimodal
110	no	no	none	none	normal	normal
118	99 %	no	none	none	unimodal	unimodal
128	no	95 %	none	none	normal	normal
138	no	no	none	none	normal	unimodal
149	no	no	none	none	normal	normal
153	99 %	no	none	none	normal	normal
156	no	no	none	none	normal	normal
163	99 %	no	none	none	unimodal	unimodal
167	no	no	one	one	unimodal	unimodal
170	no	99 %	none	none	normal	normal
177	95 %	no	none	none	normal	normal
180	no	no	none	none	normal	unimodal
183	95 %	no	none	none	normal	unimodal
187	99 %	no	none	none	normal	normal
194	99 %	no	none	none	normal	unimodal
196	no	95 %	one	none	normal	normal

Table 2. Detailed overview of outliers mentioned in Table 1.

PCB	Storage condition	Time	High / Low outlier	Individual result / Bottle mean	Unit #
28	18 °C	2 weeks	high	bottle mean	667
52	18 °C	2 weeks	high	individual result	667
52	18 °C	2 weeks	high	bottle mean	667
74	18 °C	1 week	low	bottle mean	1569
95	18 °C	4 weeks	low	individual result	1726
95	18 °C	2 weeks	high	individual result	667
167	18 °C	4 weeks	high	individual result	1726
167	18 °C	4 weeks	high	bottle mean	1726
196	18 °C	4 weeks	high	individual result	1726

Distributions of individual results and bottle means were normal or at least unimodal for all congeners.
From this descriptive evaluation it was concluded that using all measurement data available for ERM-BB350 would result in reliable estimates for the uncertainty of the between-bottle homogeneity for all congeners.

4.1.2 Estimation of uncertainty of homogeneity of ERM-BB350

Estimation of uncertainty of between-bottle homogeneity is most easily done by analysis of variance (ANOVA), which can separate the between-bottle variation (s_{bb}) from the within-bottle variation (s_{wb}) [17]. The latter is equivalent to the analytical variation if the individual subsamples are representative for the whole bottle. Evaluation by ANOVA is only possible if data follow normal or unimodal distributions. This condition is fulfilled, as can be seen in Table 1.
Method repeatability, expressed as relative standard deviation within-bottles (s_{wb}), is given in Equation 1:

$$s_{wb} = \frac{\sqrt{MS_{within}}}{\bar{y}} \qquad \text{Equation 1}$$

Where:
MS_{within} = mean square within a bottle from an ANOVA
$\bar{y}$ = average of all results of the homogeneity study

Between-bottle variability (s_{bb}) expressed as a relative standard deviation is given by Equation 2:

$$s_{bb} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}} \qquad \text{Equation 2}$$

Where:
$MS_{between}$ = mean square among bottles from an ANOVA
MS_{within} = mean square within a bottle from an ANOVA
n = average number of replicates per bottle

It should be noted that s_{bb} and s_{wb} are estimates of the true standard deviations and subject to random fluctuations. Therefore, the mean square between groups ($MS_{between}$) can be smaller than the mean squares within groups (MS_{within}), resulting in negative arguments under the square root used for the estimation of the between-bottle variation, whereas the natural lower limit is zero. In this case, u^*_{bb}, the maximum inhomogeneity that could be hidden by method repeatability, was calculated as described by Linsinger *et al.* [18]. The u^*_{bb} is comparable to the limit of detection of an analytical method, yielding the maximum amount

of inhomogeneity that might be undetected by the given study setup. This u^*_{bb}, expressed in relative terms, is defined in Equation 3:

$$u^*_{bb} = s_{meas} \sqrt[4]{\frac{2}{\nu_{smeas}}} \qquad \text{Equation 3}$$

Where:

u^*_{bb} = maximum heterogeneity that could be hidden by method repeatability

s_{meas} = measurement variability, expressed as $\frac{s_{wb}}{\sqrt{n}}$, with n being the number of replicates

ν_{smeas} = degrees of freedom for the determination of the relative standard deviation s_{meas}

A different approach was adopted for those measurands for which outlying bottle means were detected and an alternative measure of homogeneity was estimated. Between-bottle homogeneity was modelled as rectangular distribution limited by the highest outlying average. The alternative uncertainty using these outliers (u_{alt}) was then estimated in relative terms as given in Equation 4:

$$u_{alt} = \frac{|\text{widest outlier - study average}|}{\sqrt{3} \cdot \text{study average}} \qquad \text{Equation 4}$$

When a trend in the filling sequence was significant at least at 95 % confidence level, the alternative uncertainty was also assessed. This was done for PCBs 101, 128, 170 and 196. Here, u_{alt} was estimated using a rectangular distribution between the highest and lowest bottle average. The corrected uncertainty in those cases where there was a significant trend in the filling sequence is given in relative terms in Equation 5:

$$u_{alt} = \frac{|\text{highest result - lowest result}|}{2 \cdot \sqrt{3} \cdot \text{study average}} \qquad \text{Equation 5}$$

When a trend in the analytical sequence was technically significant, normalisation can applied to the data to compensate for this trend. After application of this normalisation, the resulting u_{hom} did not differ from the u_{hom} obtained using the standard approach. It was therefore decided not to correct for the analytical trend.
The homogeneity contribution used for the calculation of the final uncertainty of the material was therefore dependent on whether a trend was present in the filling sequence or whether outliers were observed among the bottle averages. All these data are shown in Table 3.

Although the observed trends in the filling sequence are statistically significant (Table 1), the relevance of these trends can be questioned. The RSD on the bottle average for those congeners for which a significant trend in the filling sequence was observed was below 0.5 %. Further, the physicochemical characteristics of the various congeners do not differ to such an extent that a de-mixing or contamination of some congeners during processing can be deemed possible. Finally, it can also be concluded that the outlying bottle averages for PCBs 28, 52, 74, and 167 are rather results of analytical artefacts and do not reflect the real distribution of these measurands in the material. For example, the outlying bottle average for PCB 167 is fully due to one analytical outlying result. No technical reasons could be found to eliminate any result and thus all outliers were retained.

Table 3. Results of the assessment of homogeneity for ERM-BB350.

PCB	s_{wb} [%]	s_{bb} [%]	u^*_{bb} [%]	u_{alt} [%]	u_{hom} [%]
28	2.1	n.c.	0.6	1.5 [1]	1.5
52	0.8	n.c.	0.2	0.6 [1]	0.6
74	2.8	n.c.	0.8	1.9 [1]	1.9
95	0.8	n.c.	0.2		0.2
99	0.4	0.2	0.1		0.2
101	0.5	n.c.	0.1	0.2 [2]	0.2
105	0.6	0.3	0.2		0.3
110	1.3	n.c.	0.4		0.4
118	0.5	0.1	0.1		0.1
128	0.5	0.3	0.1	0.4 [2]	0.4
138	0.7	n.c.	0.2		0.2
149	0.7	0.4	0.2		0.4
153	0.5	0.2	0.2		0.2
156	0.6	0.2	0.2		0.2
163	0.8	n.c.	0.2		0.2
167	3.9	0.6	1.2	4.2 [1]	4.2
170	0.8	n.c.	0.2	0.4 [2]	0.4
177	1.0	n.c.	0.3		0.3
180	0.6	n.c.	0.2		0.2
183	0.9	n.c.	0.3		0.3
187	0.9	n.c.	0.3		0.3
194	1.1	n.c.	0.3		0.3
196	0.9	n.c.	0.3	0.5 [2]	0.5

U_{alt} was calculated when outlying bottle means ([1]) or trends in filling sequence ([2]) were observed, thus ensuring that the trends and outlying values are incorporated in the homogeneity assessment; n.c.= cannot be calculated as $MS_{between} < MS_{within}$; u_{hom} lists the values that are be used for the final uncertainty assessment of the material.

The good repeatability of the measurements allows setting very tight limits for potential heterogeneity. The frequent occurrence of $MS_{between} < MS_{within}$ (n.c. in Table 3) demonstrates that material heterogeneity is smaller than can be detected for many congeners. Even with retention of all outliers, the studies demonstrated that the potential between-unit variation is generally below 1 %. Some exceptions are noted for PCB 28 (1.5 %), PCB 74 (1.9 %), and PCB 167 (4.2 %), values which were all calculated including the presence of outliers. This between-unit variation is small enough compared to the method variability usually observed (average around 4 % was obtained by the participants of the characterisation measurements for those 3 congeners). This material is therefore sufficiently homogeneous to be suitable as reference materials.

4.2 Within-bottle homogeneity and minimum sample intake

Within-bottle homogeneity is closely correlated to the minimum sample intake. Due to the intrinsic heterogeneity, individual subsamples of a material will not contain the same amount of analyte. The minimum subsample that is representative for the complete bottle is the minimum sample intake. The larger the intrinsic heterogeneity is, the larger the minimum sample intake will be. The intrinsic heterogeneity for true solutions is known to be very small to negligible.

All homogeneity and stability experiments were performed using a 70 mg sample intake (which corresponds to around 100 µL). This sample intake (and the associated precision of most analytical balances at this level)gives acceptable homogeneity results.
This minimum sample intake of 70 mg was confirmed by the results of the characterisation study, using the method information supplied by the participants. The smallest sample intake that still yields an acceptable repeatability and accuracy was taken as confirmation of the minimum sample intake of 70 mg.
This minimum sample intake was therefore set at 70 mg.

5 Assessment of stability

Stability testing is necessary to establish conditions for dispatch (short-term stability) to the customers as well as conditions for storage (long-term stability). All stability studies were conducted as isochronous stability studies [19]. In this type of studies, samples are stored for a certain interval at the test conditions. After that time, samples are moved to conditions where further degradation is negligible ("reference condition"), effectively "freezing" the degradation status of the materials. This setup allows analysis of materials of various exposure times under repeatability conditions, thus greatly improving the sensitivity of the study to detect degradation. Time, temperature and light (UV-radiation) were regarded as the most relevant influences on stability of the material. The influence of UV-radiation was minimised by the choice of an amber glass container, which eliminates most of the incoming light. In addition, ERM-BB350 is stored and dispatched in the dark, thus practically eliminating the possibility of degradation by UV-radiation. Therefore, only the influences of time and temperature needed to be investigated. PCBs themselves are chemically stable compounds. Their high boiling points (up to above 350 °C) indicate that they are not subject to thermal degradation at temperatures encountered during everyday life [2]. Therefore, the stability assessment of the material will be driven mostly by the methodology used rather than by the actual stability and the associated uncertainty is rather conservative.

5.1 Stability during transport

For the short-term stability study, samples were stored for up to 4 weeks at 18 °C and 60 °C. Reference conditions were defined as storage at 4 °C in the dark. Samples were stored at these conditions from the time zero of this study. Two bottles were stored at each temperature for 1, 2 and 4 weeks (12 ampoules). Additionally 2 ampoules were stored at the reference conditions (14 ampoules in total). After the indicated storage periods, the samples were transferred to storage at 4 °C until analysis. After the end of the study, three analyses of all certified congeners were performed on each of the 14 samples. All analyses were performed under repeatability conditions by means of a validated analytical method comprising SPE and GC/EI-MS. Sample intake was 70 mg. Details of the method used for stability testing are given in Annex B.

The results were evaluated individually for each temperature. Results were screened for single and double outliers by applying the Grubbs test at confidence levels of 95 % and 99 %, respectively. Data were plotted against time and the regression lines were calculated to check for significant trends (degradation, enrichment) due to shipping conditions. The observed slopes were tested for significance using a *t*-test, with $t_{\alpha,\,df}$ being the critical *t*-value (two-tailed) for a confidence level $\alpha = 0.05$ (95 % level of confidence). These results are summarised in Table 4.

Table 4. Results of the evaluation of the short-term stability study for ERM-BB350. If a significant slope could be observed in the data, the level of significance is given.

	18 °C			60 °C		
PCB	**Outliers**	**Slope**	u_{sts} **[%/week]**	**Outliers**	**Slope**	u_{sts} **[%/week]**
28	none	no	0.28	none	no	0.28
52	one	no	0.14	none	no	0.07
74	none	no	0.43	none	no	0.29
95	none	no	0.13	none	no	0.07
99	none	no	0.06	none	no	0.07
101	none	no	0.06	none	no	0.06
105	none	no	0.11	none	no	0.08
110	none	no	0.19	none	no	0.13
118	none	no	0.05	none	no	0.06
128	none	no	0.09	none	no	0.07
138	none	no	0.10	none	no	0.08
149	none	no	0.12	none	no	0.11
153	none	no	0.07	none	no	0.07
156	none	no	0.09	none	no	0.07
163	none	no	0.10	none	no	0.10
167	one	no	0.76	none	no	0.26
170	none	no	0.10	none	no	0.10
177	none	no	0.13	none	no	0.12
180	none	no	0.07	none	no	0.10
183	none	no	0.14	none	no	0.11
187	none	no	0.12	none	no	0.11
194	none	no	0.14	none	no	0.14
196	none	no	0.14	none	no	0.09

One outlier (individual results; single Grubbs test) was detected for PCB 52 and 167 after storage at 18 °C. No technical reason for exclusion could be found. The resulting uncertainty contributions for short-term stability were calculated according to [20] and were negligible for all congeners (between 0.06 and 0.76 %). Neither could any significant slope be observed when assessing the whole data-set, therefore the whole data-set was kept to calculate the respective uncertainty contribution from short-term stability (u_{sts}).
No statistically significant slopes were detected at 95 % level of confidence. Thus, it can be concluded that these analytes are stable at 18 °C as well as at 60 °C. Because the potential degradation due to dispatch can be considered negligible, the uncertainty contribution from the short-term stability was not considered for the estimation of the total uncertainty of the material. The results also indicate that no special requirements are needed during sample shipment to the customer.

5.2 Stability during storage

For the long-term stability study, samples were stored for up to 12 months at 18 °C [19]. Reference conditions were defined as storage at 4 °C in the dark. Samples stored at these conditions form the time zero of this study. Two bottles were stored for 0, 4, 8 and 12 months. The samples were chosen using a random stratified sample picking scheme. After the indicated storage periods, the samples were transferred to storage at 4 °C until analysis.

After the end of the study, three analyses of all certified congeners were performed under repeatability conditions by GC/EI-MS analysis on each of the 8 bottles.

Results were screened for single and double outliers by applying the Grubbs test at confidence levels of 95 % and 99 %, respectively. Data were plotted against time and the regression lines were calculated to check for significant trends (degradation, enrichment) due to storage condition. The observed slopes were tested for significance using a *t*-test, with $t_{\alpha, df}$ being the critical *t*-value (two-tailed) for a confidence level $\alpha = 0.05$ (95 % level of confidence).

No outliers were detected in the dataset. One significant trend could be seen for PCB 180 at a 95 % significance level. This trend was however not significant at 99 % confidence level. Based on the following arguments, the observed trend was considered as a statistical artefact rather than a stability issue: (i) the difference between the lowest and highest measurement for PCB 180 throughout the dataset was only 3.5 % (whereas the repeatability of the method was 0.4 %), (ii) no significant trends could be observed for none of the other congeners, while there is no reason to assume that the stability of PCB 180 should be different, (iii) the associated 36 month u_{lts} for this congener was only 1.23 % (Table 5), (iv) no trend could be found for PCB 74, while the associated 36 month u_{lts} for this congener was as high as 3.13 % (Table 5).

Due to intrinsic variation of measurement results no study can rule out degradation completely, even in the absence of statistically significant trends. It is therefore necessary to quantify the potential degradation that could be obscured by the method repeatability, i.e. to estimate the uncertainty of stability. This means, even under ideal conditions, the outcome of a stability study can only be "degradation is 0 ± x % per time". In line with [18], this uncertainty component was estimated as uncertainty of the slope of the regression line multiplied with chosen shelf life (in this case 36 months).
The results of the long-term stability assessment are summarised in Table 5. Graphical representation of the long-term stability results are given in Annex D. The results show that the material appears stable at 18 °C. Uncertainties of stability of the certified congeners during storage range from 0.74 to 3.13 % (based on a projected 36 months shelf-life). These uncertainties are taken up in the final uncertainties of the certified values. The shelf life might be revised in the future, based on the results of the stability monitoring after certification.

Table 5. Results of the evaluation of the 12 month long-term stability study for ERM-BB350 at 18 °C. The given u_{lts} is the projected estimate based on a 36 months shelf life. If a significant slope could be observed in the data, the level of significance is given.

PCB	Outliers	Slope	u_{lts} [%/36 months]
28	none	no	1.64
52	none	no	2.26
74	none	no	3.13
95	none	no	0.97
99	none	no	0.88
101	none	no	0.77
105	none	no	1.30
110	none	no	1.41
118	none	no	0.74
128	none	no	1.13
138	none	no	1.28
149	none	no	1.04
153	none	no	0.79
156	none	no	1.31
163	none	no	4.03
167	none	no	1.44
170	none	no	1.18
177	none	no	1.43
180	none	95 %	1.23
183	none	no	1.75
187	none	no	1.54
194	none	no	1.76
196	none	no	1.76

6 Characterisation

6.1 Design of the study

Guiding principle of the characterisation study was randomisation of laboratory bias. To achieve this, several laboratories were selected to perform independent analyses. Furthermore, different methodologies were employed whenever possible to confirm the absence of method bias. Laboratories were therefore free to select a method of their choice. Due to the nature of the analytes however, all participants used GC-based methods for the measurements. Participants in the characterisation study were selected based on criteria that comprised both technical aspects (# congeners measured, analytical method used) as well as aspects regarding quality management (proven competence of the requested analysis through accreditation and/or ring trial results and measurement uncertainty of the results). All selected methods were validated and the majority of the participants were accredited for PCB-analysis (Section “2 Participants")

Fulfilment of the quality management requirements ensured the technical competence of the participants. Most laboratories are accredited to ISO 17025, although in some cases not all congeners were covered by the scope of their accreditation. Where measurements are covered by the scope of accreditation, the accreditation number is stated in the list of participants (Section "2 Participants"). Non-accredited participants submitted all requested documents in order to assess their competence.

The certification exercise was performed in 2009. Thirteen laboratories were carefully selected to perform the measurements. Samples for the characterisation study covered the whole batch and were selected using a random stratified sampling scheme. Laboratories received a quality control sample (BCR-350) as an unknown to demonstrate trueness of their results. Laboratories were requested to report the results on this material together with the results on the candidate CRM ERM-BB350. The measurement was set-up as follows:

- Three independent analyses of 2 units of ERM-BB350 were performed per laboratory. The analyses of one unit were spread over at least two days, to ensure within-laboratory reproducibility conditions.
- Independent calibration curves were prepared for the measurements of each day. No common calibrants were provided.
- Laboratories performed and reported results for method blanks.
- Laboratories measured a blind control sample (BCR-350).

Laboratories were also requested to give estimations of the expanded uncertainties of the mean value of the six results. No approach for the estimation was prescribed. The results of the quality control sample were evaluated using ERM Application Note 1 [21].

6.2 Methodology used by the participants

All except 3 laboratories used in-house methods. Three participants used a standard method (Lab Nos. 4, 5 and 14). All analytical techniques used were gas chromatography (GC) based. Sample pre-treatment varied between the laboratories; most laboratories dissolved the oil in an organic solvent before applying a clean-up step (sulphuric acid, acidified silica, deactivated aluminium oxide, deactivated silica, deactivated aluminium oxide, carbon fractionation, deactivated Florisil®, basic silica, C-18 based solid phase extraction (SPE) and dedicated PCB SPE clean-up cartridges). The details of the methods that were used for the sample pre-treatment are given in Annex E.
Individual PCBs were quantified using GC coupled to electron capture detection (GC/ECD; 5 laboratories), coupled to electron ionisation low-resolution mass spectrometry (GC/EI-LRMS; 4 laboratories) and electron ionisation high-resolution mass spectrometry (GC/EI-HRMS; 4 laboratories). The details of the instrumental methods that were used are given in Annex F.

6.3 Technical evaluation of the results

After receipt of 13 data sets, the results were subject to technical evaluation. The results of the analysis of daily method blanks assured that the trace background contamination at the laboratories was insignificant and under control. All laboratories received a bottle of BCR-350 as labelled as "fish oil quality control sample". The results on this sample could therefore directly be used to check for absence of significant bias. This material has a certified PCB content for PCB congeners 52, 101, 118, 153 and 180.
To compare the reported mass fractions with the certified values, ERM Application Note 1 was used [21]. Therefore information is needed regarding the measurement uncertainty of the laboratory. In those cases where the measurement uncertainty was clearly underestimated by the laboratory (e.g. expanded uncertainty of 3 %), a more realistic expanded measurement uncertainty of 20 % (based on experience with inter-laboratory exercises) was considered acceptable. Based on the outcome of the evaluation of the results reported for the control samples, 2 datasets were excluded based on technical reasons:

- Laboratory 1 reported values that were not in agreement with the certified values for PCBs 52 and 118, although the reported expanded measurement uncertainty for these congeners was 30 and 25 %, respectively.
- Laboratory 9 reported values that were not in agreement with the certified values for PCBs 118 and 153, although the reported expanded measurement uncertainty for these congeners was 24 and 21 %, respectively.

In all other cases where a statistically significant bias was found between the measured and certified value of the control sample, this difference was due to an underestimation of the measurement uncertainty:

- Laboratory 5: bias for PCB 153
- Laboratory 6: bias for PCB 118 and PCB 153
- Laboratory 13: bias for PCB 153

For the evaluation of the results, a more realistic estimate of the expanded measurement uncertainty of 20 % was assumed. At this measurement uncertainty, no bias was present for labs 5, 6 and 13 and congeners and therefore all those results were retained.

Apart from the exclusion of the 2 complete datasets of labs 1 and 9, selected individual results were also excluded based on technical reasons:

- Laboratory 2 reported a mistake during the preparation of the standard solution of PCB 167 and retracted the result. This result of PCB 167 was excluded.
- Laboratory 3 reported a possible co-elution for PCB 52. This was in agreement with the biased (but within the expected uncertainty) value that was reported for this congener in the control sample. This result of PCB 52 was excluded.
- Laboratory 3 made use of a GC-system on which co-elution of PCB 138 and 163 is typical [22]. A co-elution of those 2 congeners can therefore not be ruled out. The result of PCB 138 was therefore excluded.
- Laboratory 7 reported a co-elution for PCB 138. This was in agreement with the biased (but within the expected uncertainty) value that was reported for this congener in the control sample. On the GC-system that was used by the laboratory, a co-elution between PCB 138 and 163 is typical [22]. This result of PCB 138 was therefore excluded.
- Laboratory 7 also reported a problem regarding recovery for some samples. It was confirmed that this problem only affected results of those congeners for which no matching mass-labelled internal standard was used. Therefore, only those congeners for which such a standard was used were retained, all others were excluded.

An overview of all data is given in Table 6.

Table 6. Overview of evaluated datasets.

	Lab number													Accepted
PCB	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**13**	**14**	**datasets**
28	X	√	√	√	√	√	√	√	X	√	√	√	√	11
52	X	√	X	√	√	√	√	√	X	√	√	√	√	10
74	X	√	N	N	N	√	X	√	X	√	√	√	N	6
95	N	√	N	N	N	√	N	√	X	N	√	√	N	5
99	X	√	N	N	√	√	X	√	X	√	√	√	N	7
101	X	√	√	√	√	√	√	√	X	√	√	√	√	11
105	X	√	√	N	√	√	√	√	X	√	√	√	√	10
110	X	√	√	N	N	√	X	√	X	√	√	√	N	7
118	X	√	√	√	√	√	√	√	X	√	√	√	√	11
128	X	√	√	N	√	√	X	√	X	√	√	√	N	8
138	X	√	X	√	√	√	X	√	X	√	√	√	√	9
149	X	√	√	N	√	√	X	√	X	√	√	√	N	8
153	X	√	√	√	√	√	√	√	X	√	√	√	√	11
156	X	√	√	N	√	√	√	√	X	√	√	√	√	10
163	N	√	N	N	√	N	N	√	X	N	√	√	N	5
167	X	X	N	N	√	√	√	√	X	N	√	√	√	7
170	X	√	√	N	√	√	X	√	X	√	√	√	√	9
177	N	√	N	N	N	√	N	√	X	N	√	√	N	5
180	X	√	√	√	√	√	√	√	X	√	√	√	√	11
183	X	√	√	N	N	√	X	√	X	N	√	√	N	6
187	X	√	√	N	N	√	X	√	X	√	√	√	N	7
194	X	√	√	N	N	√	√	√	X	√	√	√	N	8
196	X	√	N	N	N	√	N	√	X	N	√	√	N	5

N: not measured; X: Excluded; √: Accepted;

The accepted sets of results were submitted to the following statistical tests:

- Scheffe's multiple *t*-test to check if the means of two labs are significantly different
- Dixon's test to detect outlying lab means
- Nalimov *t*-test to detect outlying lab means
- Grubb's test to detect single and double outliers
- Skewness and kurtosis test to assess the normality of the lab means distribution.

A statistically significant outlier according to the Nalimov *t*-test was identified in the dataset of PCB 52 (Annex G). However, in combination with the associated measurement uncertainty reported by laboratory # 10 (i.e. 16 %, which is similar to the reported uncertainties of the other laboratories), the measured value is not significantly different from the certified value. The statistical difference of the Nalimov *t*-test is therefore considered as a statistical artefact.

Additional material information could be defined for PCBs 95, 163 and 167. These congeners could not be certified due to a method dependent bias. Also PCBs 128 and 170 were added to the fish oil during production, but these congeners could not be certified. Two result of the characterisation dataset of PCB 128 were not in agreement with the final consensus value. Although those results were not statistically significant outliers in the dataset, it was decided not to certify the value. A laboratory contributing to the certification measurements of a new CRM should be able to find back the certified value of the final released material. For PCB 128 this would not be the case, hence the congener was not certified. In the dataset of PCB 170, a statistically significant outlier was identified. No technical reasons could justify the exclusion of the outlier. This congener was therefore not certified.
The outcome of the statistical tests of the final considered data is summarised in Annex G. All individual results are given in Annex H.

Based on the technical and statistical evaluation of the data, certified values could be established for PCBs 28, 52, 74, 99, 101, 105, 110, 118, 138, 149, 153, 156, 177, 180, 183, 187, 194 and 196.

6.4 Certified values and their uncertainties

The certified values for ERM-BB350 are calculated as the mean of means of the accepted datasets. The standard error of the mean of means was used as an estimation of the uncertainty contribution of the characterisation exercise to the mass fractions of the PCB congeners. The standard error of the mean is calculated as the standard deviation divided by the square root of the number of accepted data sets. The combined uncertainty of the certified value includes contributions from the homogeneity (u_{hom}), long-term storage (u_{lts}) and the characterisation study (u_{char}). The relative combined uncertainty is calculated as the square root of the sum of squares of the relative uncertainties of the individual contributions, according to Equation 6:

$$u_{CRM} = \sqrt{u_{hom}^2 + u_{lts}^2 + u_{char}^2} \qquad \text{Equation 6}$$

The expanded uncertainty is calculated from the combined uncertainty by multiplying the value by the coverage factor corresponding to a level of confidence of about 95 %. Coverage factor $k = 2$ was chosen for all congeners, except for PCB 177 and PCB 196, for which $k = 2.39$ and $k = 2.66$, respectively, were applied. The coverage factors applied for PCB 177 and 196 correspond to the effective degrees of freedom calculated by the Welch-Satterthwaite formula, in consequence of the fact that the degrees of freedom of the main uncertainty contribution (u_{char}) for these two congeners were down to 4. The absolute, expanded uncertainty $U_{CRM, abs}$ is calculated by multiplying the certified value with the relative, expanded uncertainty U_{CRM}. The certified values and their uncertainties are summarised in Table 7.

Table 7. Certified values and their uncertainties of ERM-BB350.

PCB	Certified value [ng/g]	u_{hom} [%]	u_{lts} [%]	u_{char} [%]	U_{CRM} [%]	U_{CRM} [%]	$U_{CRM, abs}$ [ng/g]
28	21.3	1.5	1.6	1.3	2.6	5.2	1.1
52	37.4	0.6	2.3	1.8	2.9	5.9	2.2
74	23.0	1.9	3.1	1.8	4.1	8.2	1.9
99	62	0.2	0.9	4.0	4.1	8.2	6
101	111	0.2	0.8	1.6	1.8	3.6	5
105	25.8	0.3	1.3	3.8	4.1	8.1	2.1
110	54.1	0.4	1.4	2.0	2.5	5.0	2.8
118	84	0.1	0.7	2.1	2.2	4.4	4
138	137	0.2	1.3	3.1	3.4	6.7	10
149	88	0.4	1.0	4.8	5.0	9.9	9
153	220	0.2	0.8	2.3	2.4	4.8	11
156	20.1	0.2	1.3	2.8	3.1	6.2	1.3
177	25.8	0.3	1.4	2.8	3.2	7.6	2.0
180	67	0.2	1.2	2.1	2.4	4.8	4
183	22.5	0.3	1.8	3.4	3.8	7.7	1.8
187	67	0.2	1.5	2.9	3.3	6.6	5
194	23.4	0.3	1.8	2.6	3.2	6.4	1.5
196	41	0.5	1.8	5.8	6.0	16.1	7

6.5 Additional material data

This information can be used to complement the information of the certificate of this material. For PCB 95 a method bias is possibly present. Furthermore, only a limited number of datasets is available for this congener (n = 5). Values obtained for PCB 95 through ECD-based methods were higher than those obtained through MSD-based methods, possibly due to a co-elution with PCB 74. Also for PCB 167 a method bias is possibly present. For this congener also values obtained through ECD-based methods were higher than those obtained through MSD-based methods. Again co-elution lies at the basis of this bias, possibly with PCB 128. The value for PCB 163 could not be certified due to the limited number of dataset and the lack of agreement between the results. All data are to be taken as informative values only. A summary of the values is given in Table 8.

Table 8. Additional material information of ERM-BB350. These results are not certified values and are informative only.

PCB	Mass fraction range [ng/g]	Number of datasets
95	38 - 47	5
163	43 - 73	5
167	17 - 27	7

7 Metrological traceability

The certified values for the mass fractions of PCBs are traceable to the SI. Participating laboratories used different commercially available calibrants, in-house gravimetrically prepared or CRMs, all traceable to the SI. As all methods employed during the characterisation were based upon gas chromatography, the measurands are operationally defined, i.e. as obtained by quantification using gas chromatographic methods. As up to 11 different sample pre-treatment and clean-up techniques have been used, independency to the sample preparation method is given.

8 Commutability

ERM-BB350 is prepared from artificially contaminated material. Since the PCBs were dissolved in the fish oil, there is no reason to assume that ERM-BB350 would behave differently from naturally contaminated fish oil samples.

9 Instructions for use

9.1 Storage conditions

The materials should be stored at + 18 °C ± 5 °C. Storage at + 4 °C might induce formation of condensed particles (Figure 1). Heating the ampoules at the prescribed temperature for the defined time should be adequate to solubilise all condensed fat particles. In isolated cases (e.g. following colder storage at the premises of the user), a longer heating time might be required to obtain a clear oil solution. This does not affect the stability of the material as long as the maximum temperature of 60 °C is respected.
However, the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of open samples.

Figure 1. Appearance of condensed lipid particles that may appear after prolonged cold storage. This sedimentation is perfectly normal and does not affect he certified values as long as the protocol is adhered to.

9.2 Safety precautions

The usual laboratory safety precautions apply.

9.3 Intended use of the material

This material is intended to be used for method performance control and validation purposes of the certified measurands. Samples should be heated for at least 20 min at 60 °C. The contents should be thoroughly mixed and it must be verified that the content of the ampoule is a clear solution with no visible particles. Allow the ampoules to cool down to + 18 °C ± 5 °C before opening and weighing the sub-sample. Immediately after opening the ampoule sub-samples of at least 70 mg need to be weighed out and the mass fractions of the PCBs calculated based on this mass.

For assessing the method performance, the measured values of the CRMs are compared with the certified values following a procedure described by Linsinger [21]. The procedure is described here in brief:

- Calculate the absolute difference between mean measured value and the certified value (Δ_m).
- Combine measurement uncertainty (u_m) with the uncertainty of the certified

value (u_{CRM}): $u_{\Delta} = \sqrt{u_m^2 + u_{CRM}^2}$

- Calculate the expanded uncertainty (U_{Δ}) from the combined uncertainty ($u_{\Delta,}$) using a coverage factor of two (k = 2), corresponding to a level of confidence of approximately 95 %
- If $\Delta_m \leq U_{\Delta}$ then there is no significant difference between the measurement result and the certified value, at a confidence level of about 95 %.

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Annexes

Annex A. Overview of PCB congeners.
Annex B. Details of the analytical method used for the homogeneity and stability measurements.
Annex C: Results of the homogeneity tests for ERM-BB350
Annex D: Graphical representation of the long-term stability tests for ERM-BB350
Annex E: Sample intakes and methods used for the sample pre-treatment and clean-up of the fish oil.
Annex F. Instrumental set-up and methods used for the determination of the mass fraction of PCBs.
Annex G. Summary of the statistical evaluation of the characterisation measurements for ERM-BB350
Annex H: Results of characterisation measurements for ERM-BB350

Annex A. Overview of PCB congeners.

PCB	IUPAC name	CAS No
28	2,4,4'-trichlorobiphenyl	7012-37-5
52	2,2',5,5'-tetrachlorobiphenyl	35693-99-3
74	2,4,4',5-tetrachlorobiphenyl	32690-93-0
95	2,2',3,5',6-pentachlorobiphenyl	38379-99-6
99	2,2',4,4',5-pentachlorobiphenyl	38380-01-7
101	2,2',4,5,5'-pentachlorobiphenyl	37680-73-2
105	2,3,3',4,4'-pentachlorobiphenyl	32598-14-4
110	2,3,3',4',6-pentachlorobiphenyl	38380-03-9
118	2,3',4,4',5-pentachlorobiphenyl	31508-00-6
128	2,2',3,3',4,4'-hexachlorobiphenyl	38380-07-3
138	2,2',3,4,4',5'-hexachlorobiphenyl	35065-28-2
149	2,2',3,4',5',6-hexachlorobiphenyl	38380-04-0
153	2,2',4,4',5,5'-hexachlorobiphenyl	35065-27-1
156	2,3,3',4,4',5-hexachlorobiphenyl	38380-08-4
163	2,3,3',4',5,6-hexachlorobiphenyl	74472-44-9
167	2,3',4,4',5,5'-hexachlorobiphenyl	52663-72-6
170	2,2',3,3',4,4',5,-heptachlorobiphenyl	35065-30-6
177	2,2',3,3',4,5',6'-heptachlorobiphenyl	52663-70-4
180	2,2',3,4,4',5,5'-heptachlorobiphenyl	35065-29-3
183	2,2',3,4,4',5',6-heptachlorobiphenyl	52663-69-1
187	2,2',3,4',5,5',6-heptachlorobiphenyl	52663-68-0
194	2,2',3,3',4,4',5,5'-octachlorobiphenyl	35694-08-7
196	2,2',3,3',4,4',5,6'-octachlorobiphenyl	42740-50-1

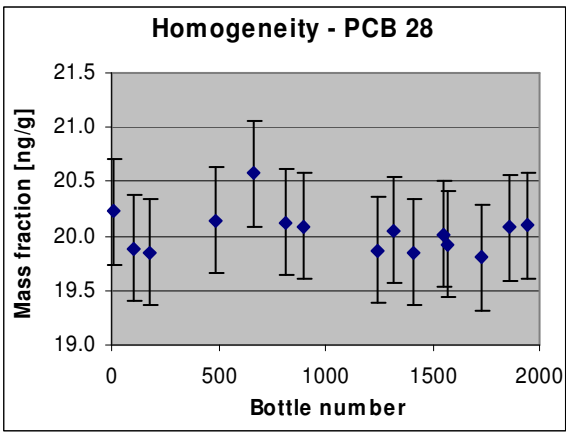
Annex B. Details of the analytical method used for the homogeneity and stability measurements.

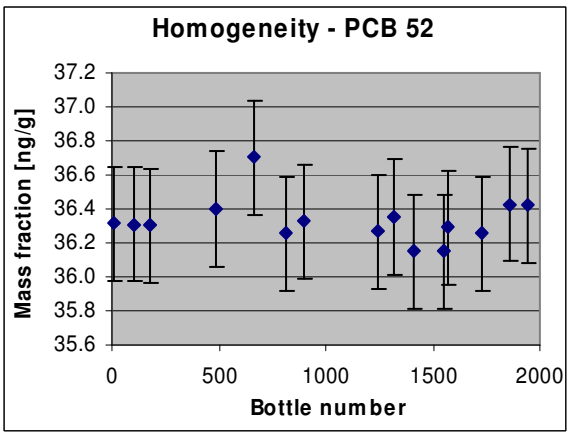
Capillary GC-column	Column dimensions	Injection type	Detector	Internal stds	# cal. points
DB-XLB (J&W)	60 m x 0.25 mm x 0.25 µm	splitless	EI-LRMS	$^{13}C_{12}$-labelled PCBs 28, 52, 101, 105, 118, 128, 138, 153, 156, 170, and 180	5

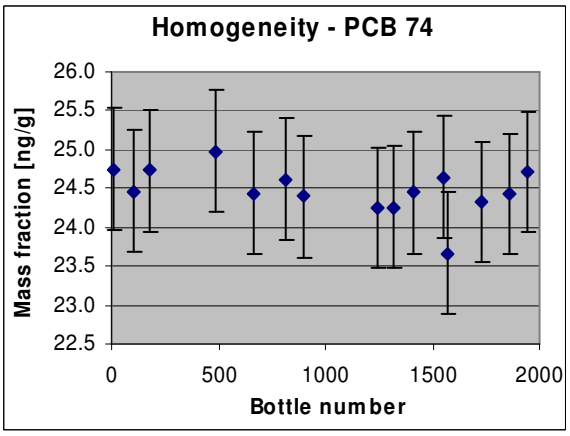
Sample intake (g)	Standard method	Extraction	Clean-up
0.07	no	dissolving in *n*-hexane	Varian BondElut PCB SPE cartridge, elution with *n*-hexane

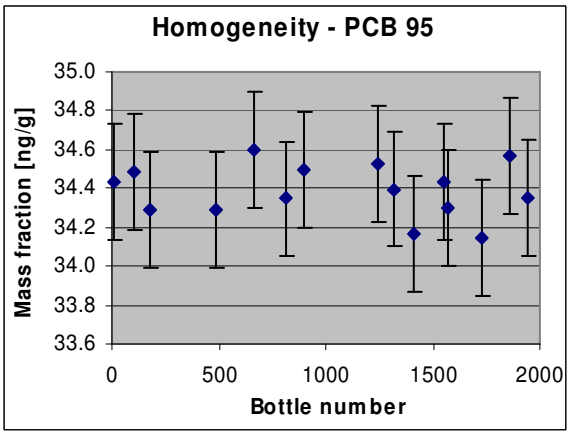
Annex C: Results of the homogeneity tests for ERM-BB350

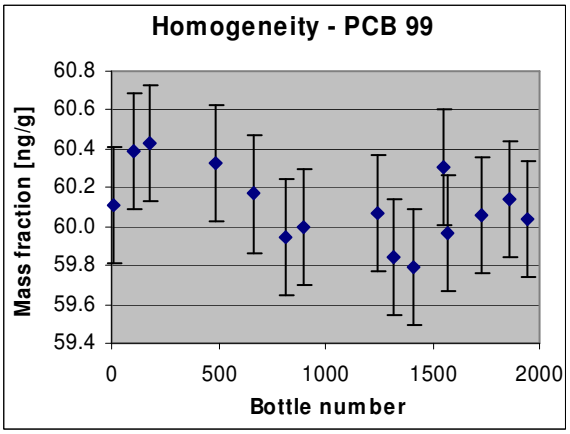
The graphs show bottle averages and their 95 % confidence intervals. Confidence intervals were based on the "within-bottle" standard deviation for each congener rather than on the standard deviation of the 3 replicates per bottle. Absolute values do not agree with the certified values, this is most likely due to different calibrations.

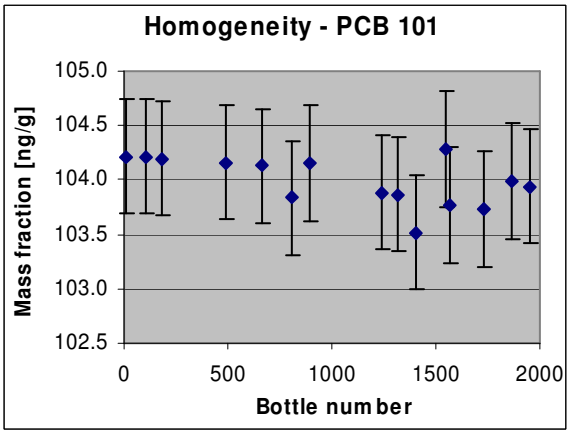


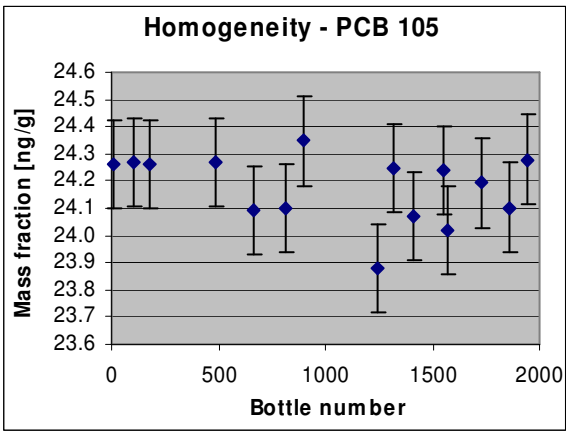


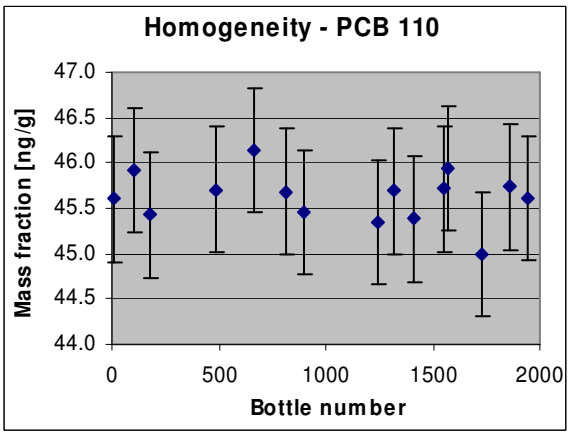









Homogeneity - PCB 105
Mass fraction [ng/g]
24.6
24.5
24.4
24.3
24.2
24.1
24.0
23.9
23.8
23.7
23.6
0
500
1000
1500
2000
Bottle number


Homogeneity - PCB 110
Mass fraction [ng/g]
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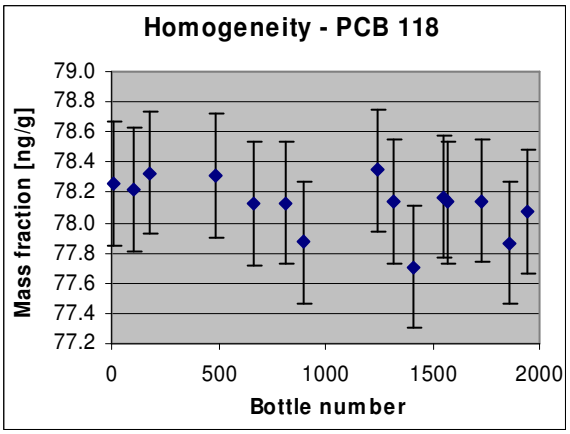
46.5
46.0
45.5
45.0
44.5
44.0
0
500
1000
1500
2000
Bottle number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Mass fraction [ng/g]
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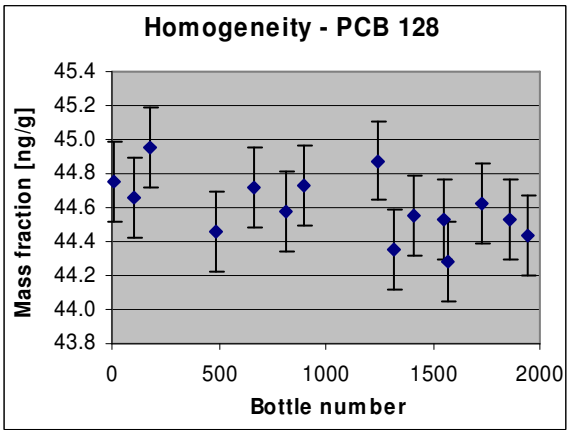
78.8
78.6
78.4
78.2
78.0
77.8
77.6
77.4
77.2
0
500
1000
1500
2000
Bottle number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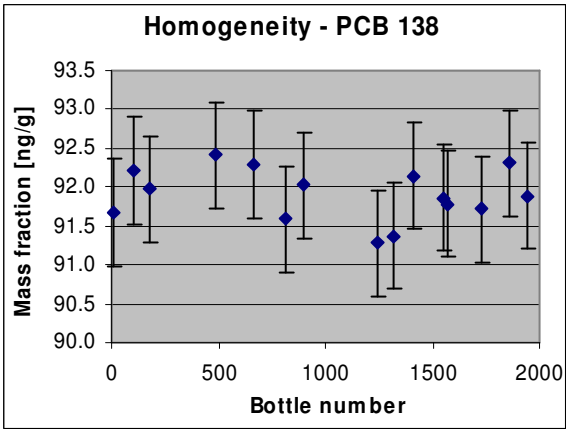
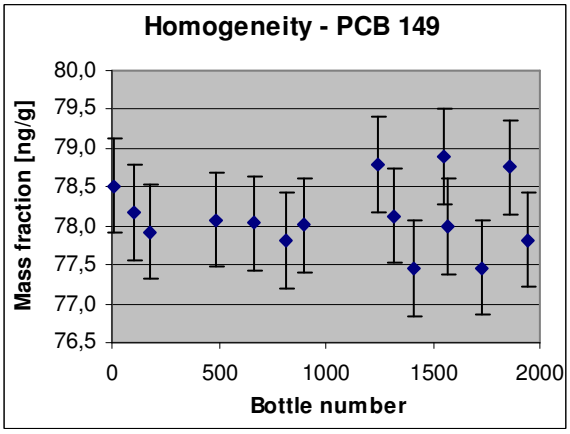
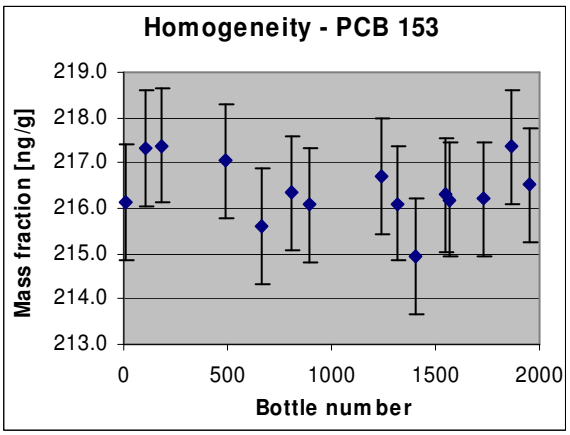
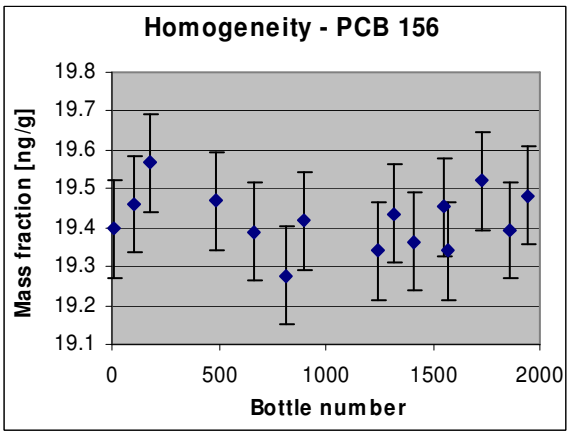
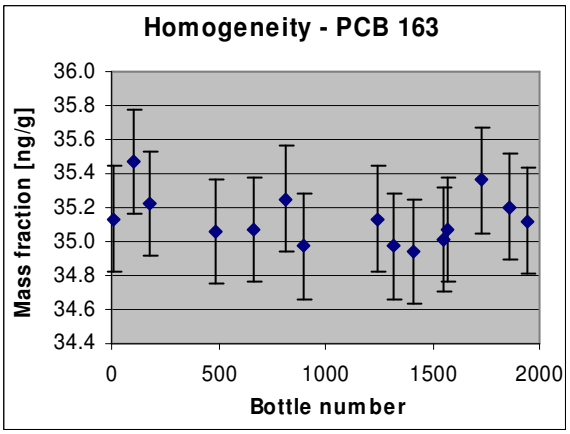
Mass fraction [ng/g]
45.4
45.2
45.0
44.8
44.6
44.4
44.2
44.0
43.8
0
500
1000
1500
2000
Bottle number

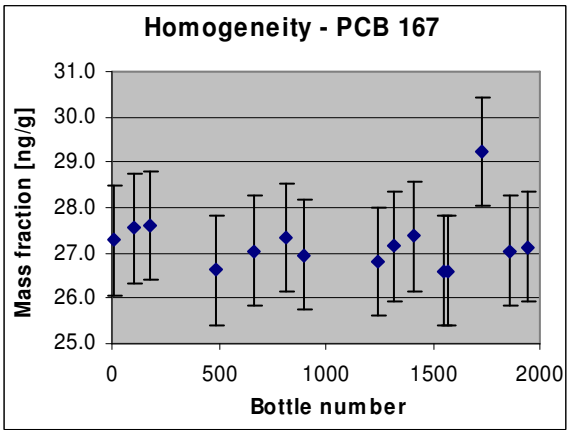

Homogeneity - PCB 138
Mass fraction [ng/g]
93.5
93.0
92.5
92.0
91.5
91.0
90.5
90.0
0
500
1000
1500
2000
Bottle number

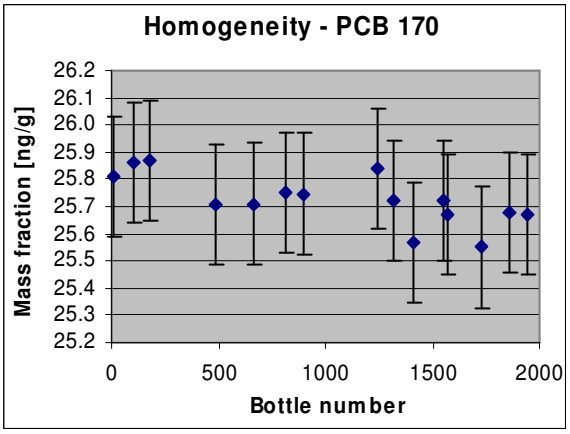

Homogeneity - PCB 149
Mass fraction [ng/g]
80,0
79,5
79,0
78,5
78,0
77,5
77,0
76,5
0
500
1000
1500
2000
Bottle number

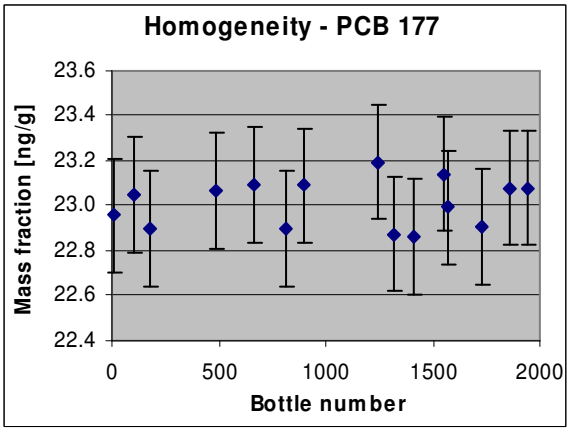

Homogeneity - PCB 153
Mass fraction [ng/g]
219.0
218.0
217.0
216.0
215.0
214.0
213.0
0
500
1000
1500
2000
Bottle number

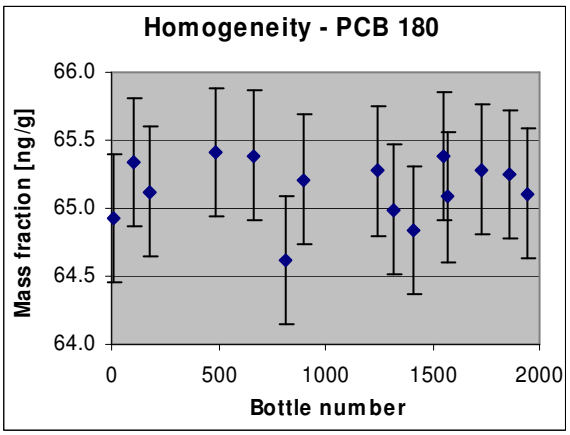

Homogeneity - PCB 156
Mass fraction [ng/g]
19.8
19.7
19.6
19.5
19.4
19.3
19.2
19.1
0
500
1000
1500
2000
Bottle number

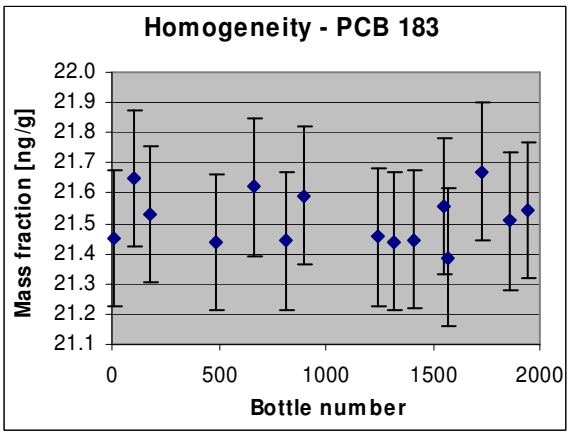

Homogeneity - PCB 163
Mass fraction [ng/g]
36.0
35.8
35.6
35.4
35.2
35.0
34.8
34.6
34.4
0
500
1000
1500
2000
Bottle number

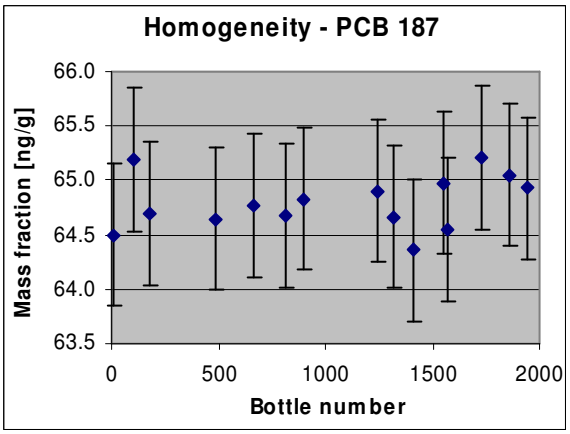

Homogeneity - PCB 167
Mass fraction [ng/g]
31.0
30.0
29.0
28.0
27.0
26.0
25.0
0
500
1000
1500
2000
Bottle number

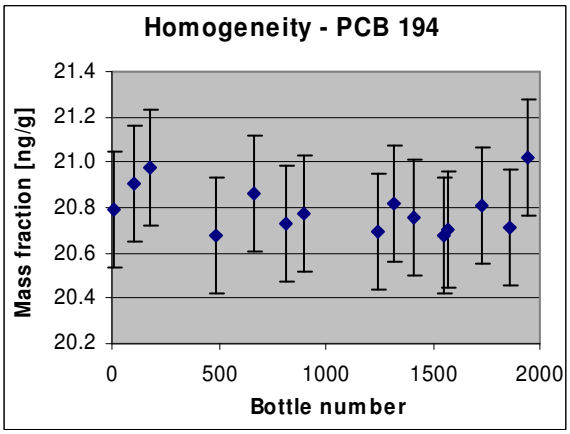

Homogeneity - PCB 170
Mass fraction [ng/g]
26.2
26.1
26.0
25.9
25.8
25.7
25.6
25.5
25.4
25.3
25.2
0
500
1000
1500
2000
Bottle number

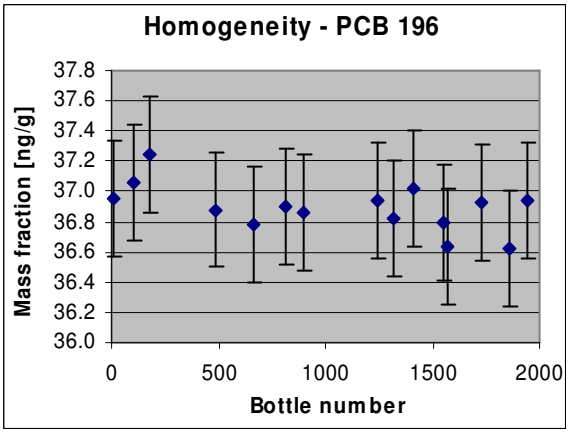

Homogeneity - PCB 177
Mass fraction [ng/g]
23.6
23.4
23.2
23.0
22.8
22.6
22.4
0
500
1000
1500
2000
Bottle number


Homogeneity - PCB 180
Mass fraction [ng/g]
66.0
65.5
65.0
64.5
64.0
0
500
1000
1500
2000
Bottle number


Homogeneity - PCB 183
Mass fraction [ng/g]
22.0
21.9
21.8
21.7
21.6
21.5
21.4
21.3
21.2
21.1
0
500
1000
1500
2000
Bottle number

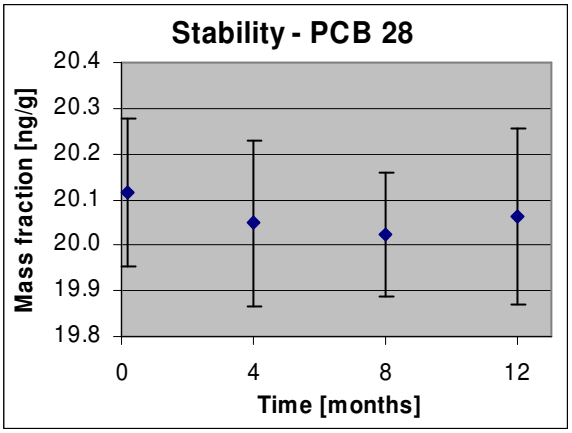

Homogeneity - PCB 187
Mass fraction [ng/g]
66.0
65.5
65.0
64.5
64.0
63.5
0
500
1000
1500
2000
Bottle number

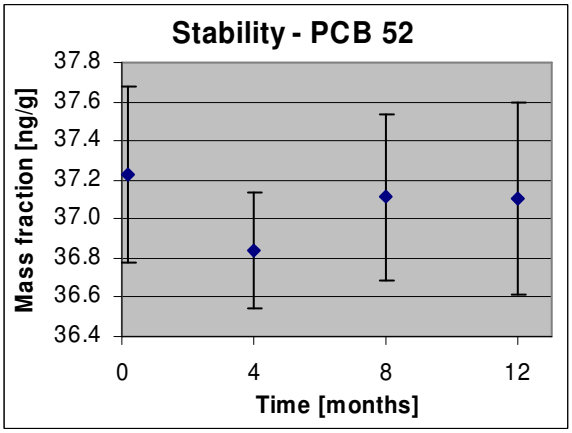

Homogeneity - PCB 194
Mass fraction [ng/g]
21.4
21.2
21.0
20.8
20.6
20.4
20.2
0
500
1000
1500
2000
Bottle number

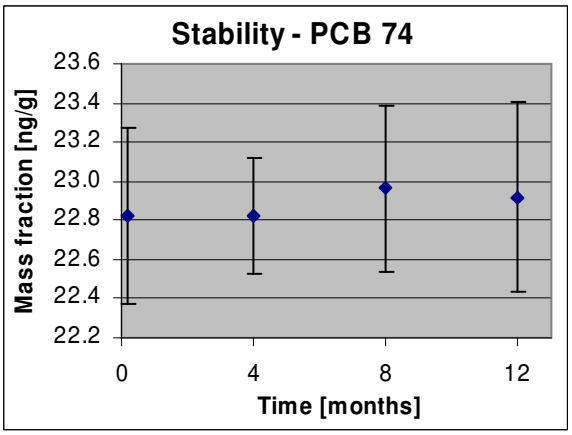

Homogeneity - PCB 196
Mass fraction [ng/g]
37.8
37.6
37.4
37.2
37.0
36.8
36.6
36.4
36.2
36.0
0
500
1000
1500
2000
Bottle number

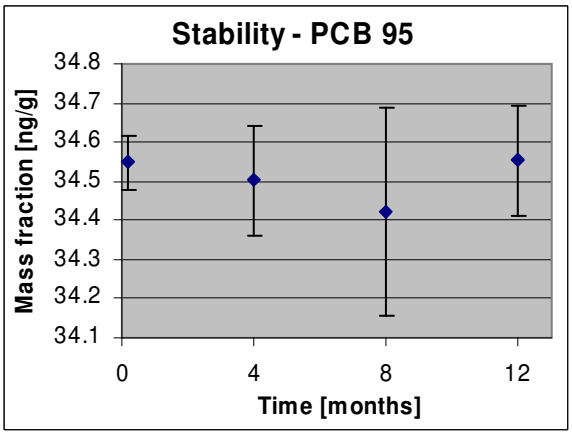
Annex D: Graphical representation of the long-term stability tests for ERM-BB350

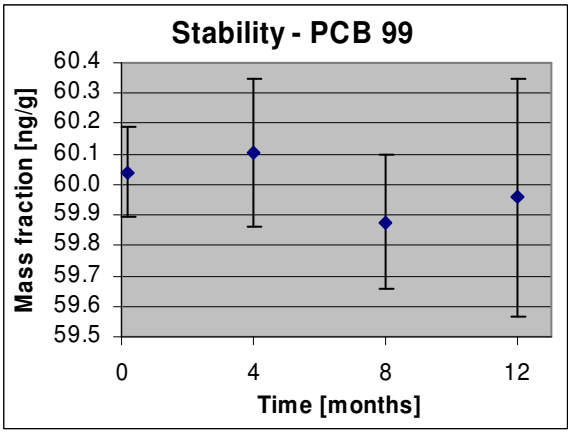
The graphs show bottle averages per time point and their 95 % confidence intervals. Confidence intervals were based on the standard deviations of the 6 replicates per time/temperature combination. Data points for the measurement at t = 0 are slightly offset to the right on the graphs to enhance the readability. Absolute values do not agree with the certified values, this is most likely due to different calibrations.

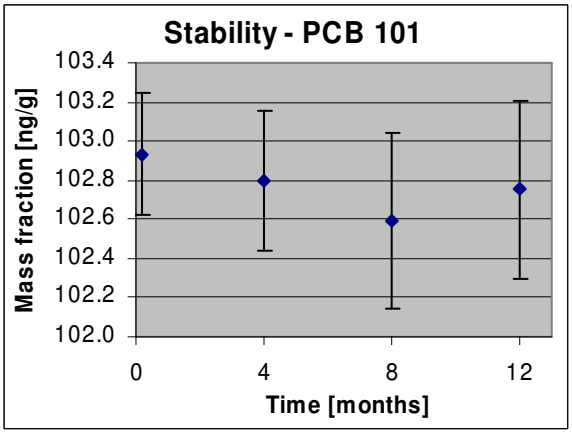


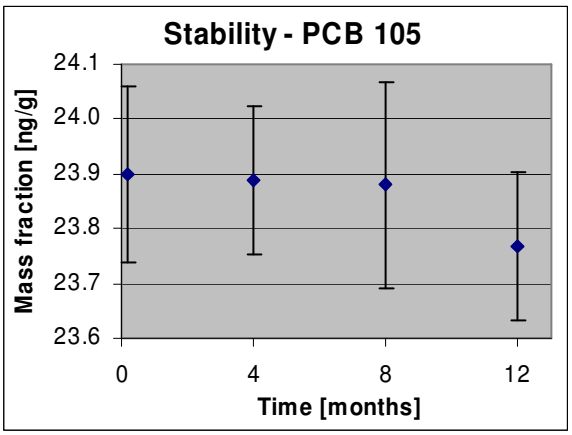


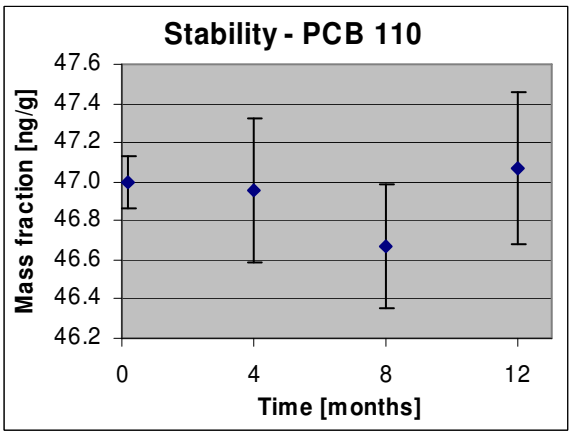


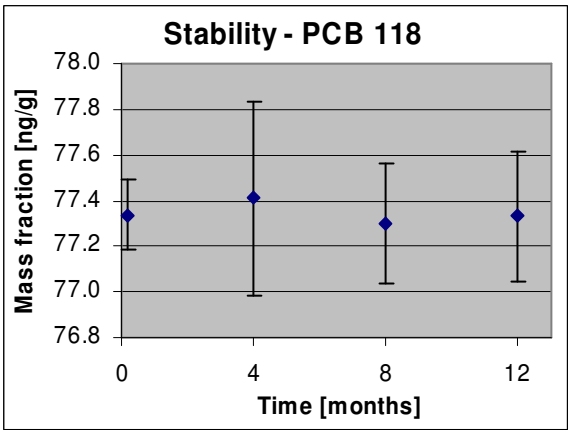


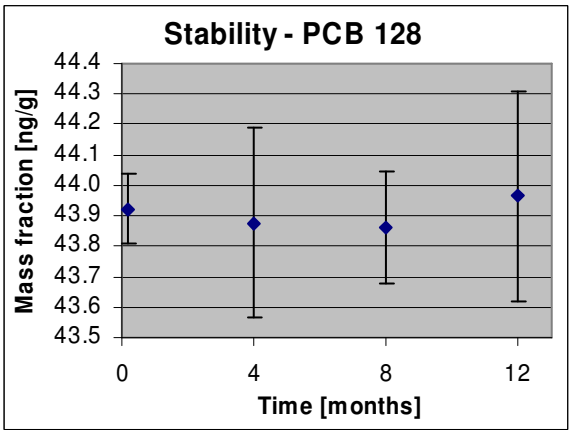


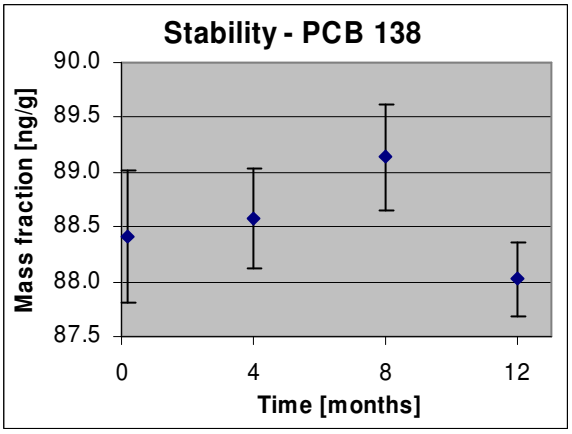


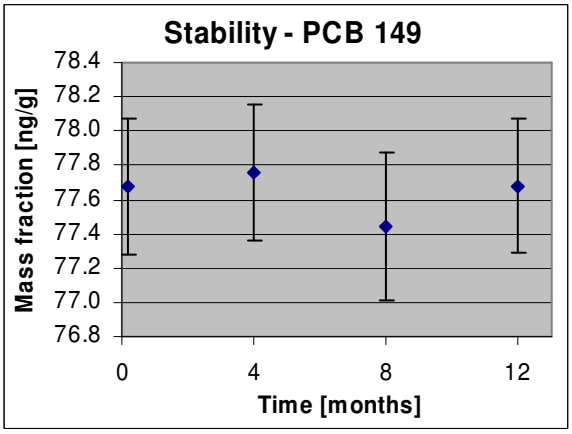

Stability - PCB 105
Mass fraction [ng/g]
24.1
24.0
23.9
23.8
23.7
23.6
0
4
8
12
Time [months]

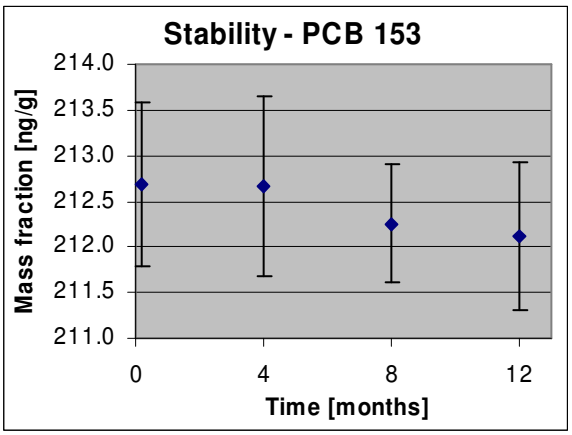

Stability - PCB 110
Mass fraction [ng/g]
47.6
47.4
47.2
47.0
46.8
46.6
46.4
46.2
0
4
8
12
Time [months]

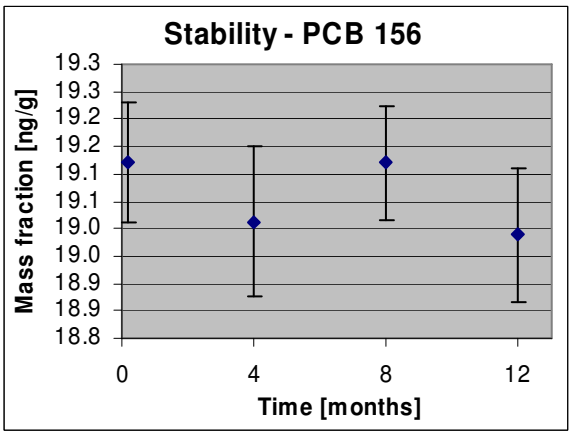

Stability - PCB 118
Mass fraction [ng/g]
78.0
77.8
77.6
77.4
77.2
77.0
76.8
0
4
8
12
Time [months]

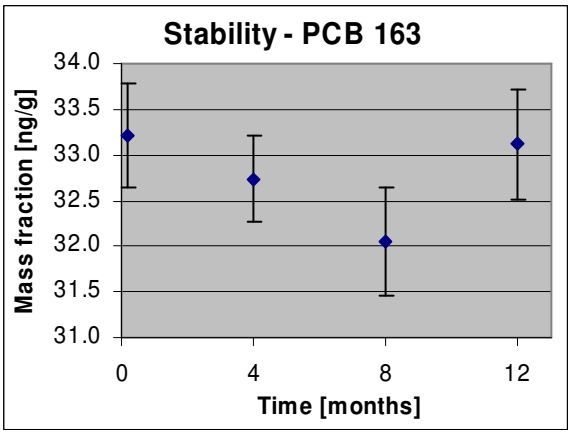

Stability - PCB 128
Mass fraction [ng/g]
44.4
44.3
44.2
44.1
44.0
43.9
43.8
43.7
43.6
43.5
0
4
8
12
Time [months]

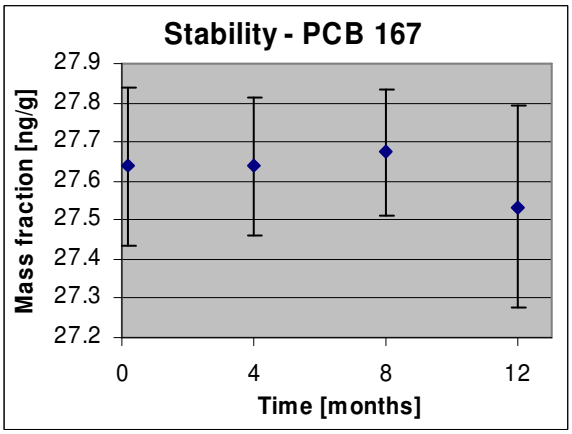

Stability - PCB 138
Mass fraction [ng/g]
90.0
89.5
89.0
88.5
88.0
87.5
0
4
8
12
Time [months]

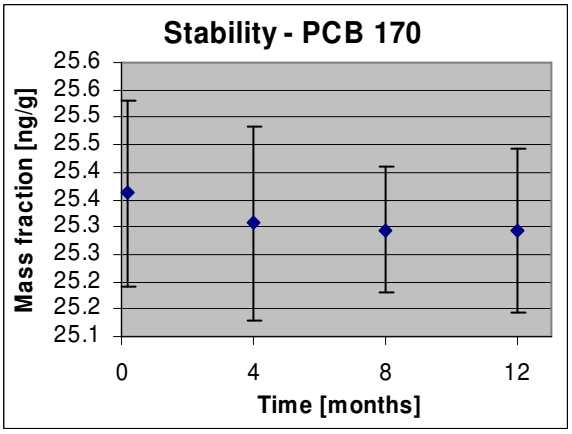

Stability - PCB 149
Mass fraction [ng/g]
78.4
78.2
78.0
77.8
77.6
77.4
77.2
77.0
76.8
0
4
8
12
Time [months]

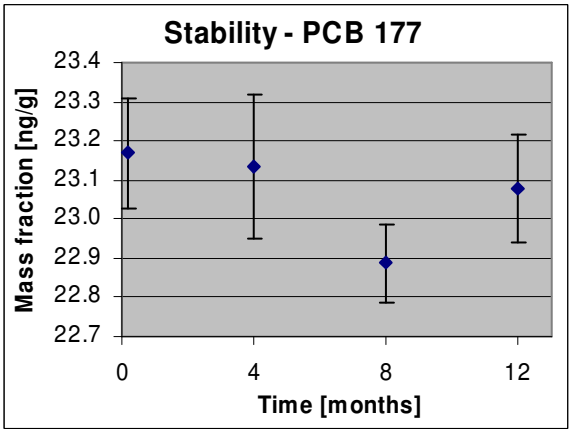

Stability - PCB 153
Mass fraction [ng/g]
214.0
213.5
213.0
212.5
212.0
211.5
211.0
0
4
8
12
Time [months]

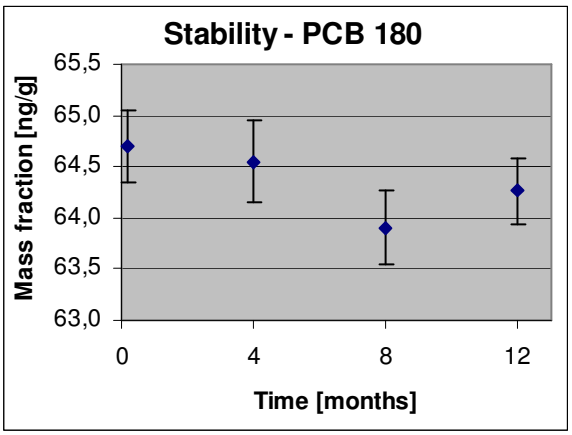

Stability - PCB 156
Mass fraction [ng/g]
19.3
19.3
19.2
19.2
19.1
19.1
19.0
19.0
18.9
18.9
18.8
0
4
8
12
Time [months]

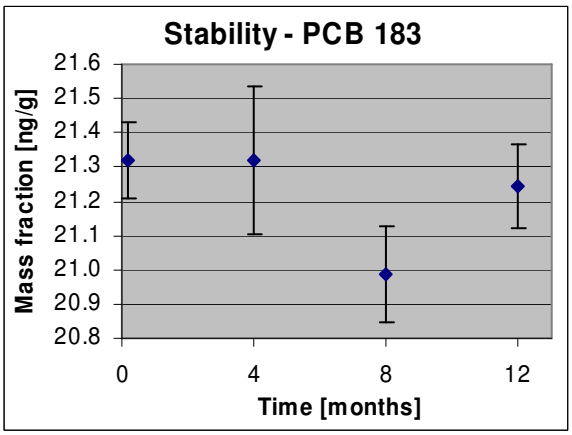

Stability - PCB 163
Mass fraction [ng/g]
34.0
33.5
33.0
32.5
32.0
31.5
31.0
0
4
8
12
Time [months]

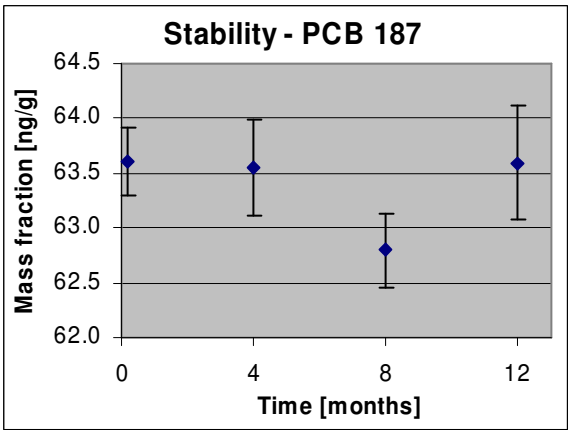

Stability - PCB 167
Mass fraction [ng/g]
27.9
27.8
27.7
27.6
27.5
27.4
27.3
27.2
0
4
8
12
Time [months]

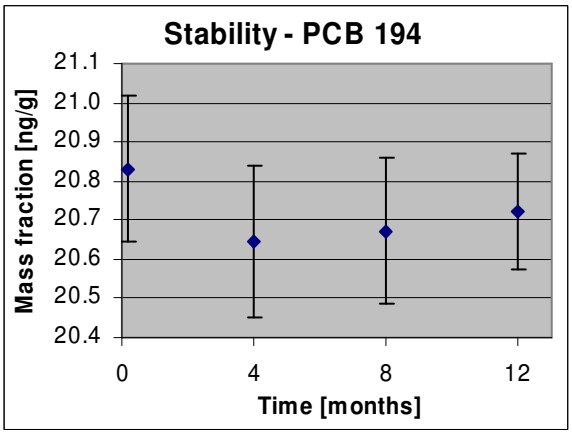

Stability - PCB 170
Mass fraction [ng/g]
25.6
25.6
25.5
25.5
25.4
25.4
25.3
25.3
25.2
25.2
25.1
0
4
8
12
Time [months]

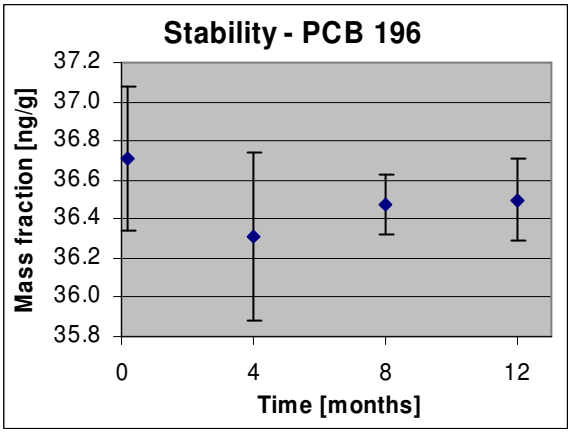

Stability - PCB 177
Mass fraction [ng/g]
23.4
23.3
23.2
23.1
23.0
22.9
22.8
22.7
0
4
8
12
Time [months]


Stability - PCB 180
Mass fraction [ng/g]
65,5
65,0
64,5
64,0
63,5
63,0
0
4
8
12
Time [months]


Stability - PCB 183
Mass fraction [ng/g]
21.6
21.5
21.4
21.3
21.2
21.1
21.0
20.9
20.8
0
4
8
12
Time [months]


Stability - PCB 187
Mass fraction [ng/g]
64.5
64.0
63.5
63.0
62.5
62.0
0
4
8
12
Time [months]


Stability - PCB 194
Mass fraction [ng/g]
21.1
21.0
20.9
20.8
20.7
20.6
20.5
20.4
0
4
8
12
Time [months]


Stability - PCB 196
Mass fraction [ng/g]
37.2
37.0
36.8
36.6
36.4
36.2
36.0
35.8
0
4
8
12
Time [months]

Annex E: Sample intakes and methods used for the sample pre-treatment and clean-up of the fish oil.

Lab No	Sample intake (g)	Standard method	Extraction	Clean-up
1	0.2	no	dissolving in *cyclo*-hexane	addition of concentrated sulphuric acid, mixing and centrifugation for 10 min at 1643 g
2	0.15	no	dissolving in *n*-hexane	8 g acidified silica (44 %; *m/m*), elution with 25 mL *n*-hexane, evaporation and resolubilisation in 150 µl *iso*-octane
3	0.5	no	dissolving in *n*-hexane	0.5 g oil was dissolved in 10 mL of *n*-hexane; 1 mL of the solution was cleaned up by column chromatography (3 g of 5 % deactivated aluminium oxide followed by 3 g of 3 % deactivated silica)
4	0.4	no	n.a.	chromatographic column filled with 9 g acidified silica, 1.5 g deactivated aluminium oxide and 0.5 g water free sodium sulphate; elution with *n*-hexane
5	0.3	conform BELAC document I014	n.a.	mixed acid silica/aluminium oxide cleanup column (6 g silica-40 % H_2SO_4 + 1 g aluminium oxide-10 % H_2O + 5 g anhydrous Na_2SO_4), eluting with 20 mL *n*-hexane
6	0.4	no	n.a	Aluminium oxide, silica gel, sulphuric acid treated
7	0.5	no	dissolving in *n*-hexane/dichloromethane	carbon fractionation, aluminium oxide and acidified silica adsorption chromatography
8	0.3	no	n.a.	sulphuric acid/silica column (40 % H_2SO_4, 20 g) followed by a fractionation with a silica column (deactivated with 1.5 % H_2O, 1.8 g)
9	0.4	no	dissolving in *n*-hexane	combined clean-up with $AgNO_3/SiO_2$ + H_2SO_4/SiO_2 + Al_2O_3
10	0.3	no	dissolving in *n*-hexane	treatment with concentrated sulphuric acid for 30 minutes, separation on 2 g Florisil® deactivated with 1.2 % water
11	0.3	no	dissolving in *n*-hexane	1st step: chromatography on column filled with acidic, basic and neutral silica, elution with *n*-hexane; 2nd step: SPE-column with C-18ec, elution with acetonitrile
13	0.07	no	dissolving in *n*-hexane	Varian BondElut PCB SPE cartridge, elution with *n*-hexane
14	0.25	EPA Method 1668, Revision A	dissolving in *n*-hexane	multilayer silica and Florisil® column

n.a.: not applicable

Annex F. Instrumental set-up and methods used for the determination of the mass fraction of PCBs.

Lab No	Capillary GC-column	Column dimensions	Injection type	Detector	Internal stds	# cal. points
1	SPB-5 (Supelco)	60 m x 0.25 mm x 0.25 µm	cold splitless with PTV injector	ECD and ECNI-LRMS	PCBs 29, 112 and 207	5
2	HT-8 (SGE)	25 m x 0.22 mm x 0.25 µm	cold splitless with PTV injector	EI-LRMS	PCB 143	6
3	DB-5 (J&W)	50 m x 0.2 mm x 0.33 µm	splitless	ECD	PCB 53	7
4	HT-8 (SGE)	25 m x 0.22 mm x 0.25 µm	splitless	EI-LRMS	Mirex	5
5	HT-8 (SGE)	50 m x 0.22 mm x 0.25 µm	splitless	EI-HRMS	$^{13}C_{12}$-labelled PCBs 28, 52, 101, 105, 118, 138, 153, 156, 167, 170, and 180	7
6	CP-Sil8-CB and CP-sil19-CB (Varian)	50 m x 150 mm x 0.2 µm	splitless	ECD	PCBs 112 and 207	6
7	DB-5 (J&W)	60 m x 0.25 mm x 0.1 µm	solvent vent	EI-LRMS	$^{13}C_{12}$-labelled PCBs 28, 52, 101, 105, 114, 118, 123, 138, 153, 156, 157, 167, 170, 180, 189, 194 and 209	single RRF
8	CP-Sil8CB/CP-Sil19CB/CP-Sil13CB (Varian)	50 m x 0.2 mm x 0.33 µm / 50 m x 0.2 mm x 0.33 µm / 50 m x 0.25 x 0.2 µm	pulsed splitless	ECD	PCBs 103 and 198	7 - 9
9	SLB5-MS (Supelco)	60 m x 0.25 mm x 0.25 µm	splitless	EI-LRMS	$^{13}C_{12}$-labelled PCBs 28, 52, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 180, 189, 194, 206, and 209	5
10	HT-8 (SGE) and DB-5 (J&W)	50 m x 0.22 mm x 0.25 µm	pulsed splitless	ECD	PCBs 29 and 112	5
11	Rtx-CLPesticides2 (Restek)	30 m x 0.25 mm x 0.2 µm	cold splitless with PTV injector	EI-HRMS	$^{13}C_{12}$-labelled PCBs 28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180, 189, and 194	5
13	DB-XLB (J&W)	60 m x 0.25 mm x 0.25 µm	splitless	EI-LRMS	$^{13}C_{12}$-labelled PCBs 28, 52, 101, 105, 118, 128, 138, 153, 156, 170, and 180	5
14	DB-XLB (J&W)	60 m x 0.25 mm x 0.25 µm	splitless	EI-HRMS	$^{13}C_{12}$-labelled PCBs 28, 52, 101, 138, 153, 180, and 209	5

Annex G. Summary of the statistical evaluation of the characterisation measurements for ERM-BB350

PCB	28	52	74	95	99	101	105	110	118	128	138
# datasets	11	10	6	5	7	11	10	7	11	8	10
# replicate measurements	66	60	36	30	42	66	60	42	66	48	60
Mean of means [ng/g]	21.28	37.43	22.96	41.72	62.49	111.42	25.84	54.12	84.20	31.72	136.95
RSD [%]	4.3	5.6	4.5	10.3	10.6	5.4	12.1	5.4	6.8	18.3	10.7
Relative standard error [%]	1.3	1.8	1.8	4.6	4.0	1.6	3.8	2.0	2.1	6.5	3.4
All datasets compatible two by two? (Scheffe's test)	No	No	No	No	No	No	No	No	No	No	No
Outlying means? (Dixon test)	No	No	No	No	No	No	No	No	No	No	No
Outlying means? (Nalimov *t*-test)	No (0.01)	Yes	No	No	No	No	No	No	No (0.01)	No	No
Outlying means? (Grubbs test)	No	No (0.01)	No	No	No	No (0.01)	No	No	No	No	No
Distribution of means normal? (Skewness & kurtosis)	Yes	Yes (0.01)	i.d.	i.d.	Yes	Yes	Yes	Yes	Yes	Yes	Yes

i.d.: insufficient data; $\alpha = 0.05$ unless stated otherwise

Annex G. Summary of the statistical evaluation of the characterisation measurements for ERM-BB350 - Continued

PCB	149	153	156	163	167	170	177	180	183	187	194	196
# datasets	8	11	9	5	7	9	5	11	6	7	8	5
# replicate measurements	48	66	54	30	42	54	30	66	36	42	48	30
Mean of means [ng/g]	87.89	219.68	20.12	56.51	21.46	26.18	25.82	67.24	22.54	67.00	23.41	41.40
RSD [%]	13.7	7.5	8.4	23.1	17.4	10.3	6.3	6.8	8.3	7.7	7.4	12.9
Relative standard error [%]	4.8	2.3	2.8	10.3	6.6	3.4	2.8	2.1	3.4	2.9	2.6	5.8
All datasets compatible two by two? (Scheffe's test)	No	No	No	No	No	No	No	No	No	No	No	No
Outlying means? (Dixon test)	No	No	No	No	No	No	No	No	No	No	No	No
Outlying means? (Nalimov *t*-test)	No (0.01)	No	No (0.01)	No	No	Yes	No	No (0.01)	No	No (0.01)	No (0.01)	No
Outlying means? (Grubbs test)	No	No	No	No	No	No (0.01)	No	No	No	No	No	No
Distribution of means normal? (Skewness & kurtosis)	Yes	Yes	Yes	Yes	Yes	Yes	i.d.	Yes	i.d.	Yes (0.01)	Yes	i.d.

i.d.: insufficient data; $\alpha = 0.05$ unless stated otherwise

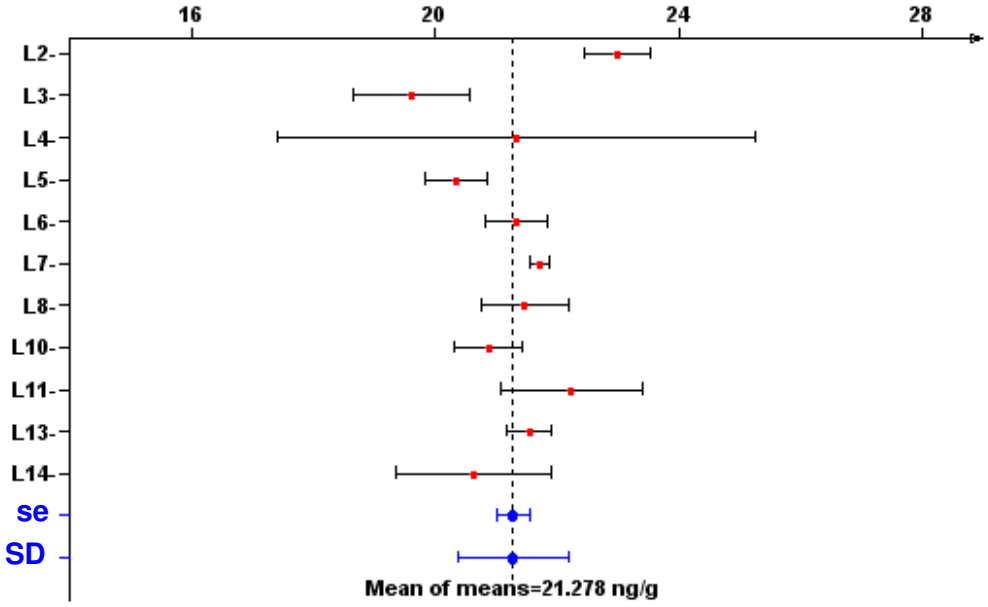
Annex H: Results of characterisation measurements

The tables in Annex H also contain the results that were excluded for technical reasons. These data are only given for informative purposes. The excluded data is presented in *italics* and the lab code is marked with an asterisk (*). The graphs in Annex H show standard deviations. Results with a low standard deviation may well have a large measurement uncertainty.

PCB 28 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
*1**	*14.30*	*13.90*	*16.80*	*15.00*	*16.90*	*16.80*	*15.62*	*1.38*
2	23.00	23.70	22.20	23.50	22.70	22.80	22.98	0.55
3	18.00	20.40	19.20	19.80	19.60	20.70	19.62	0.96
4	22.00	25.00	16.00	25.00	23.00	17.00	21.33	3.93
5	20.00	20.00	20.00	21.00	21.00	20.00	20.33	0.52
6	22.00	22.00	21.00	21.00	21.00	21.00	21.33	0.52
7	21.45	21.74	21.87	21.87	21.58	21.76	21.71	0.17
8	21.59	22.30	21.63	21.97	20.25	21.07	21.47	0.72
*9**	*16.70*	*17.20*	*15.80*	*16.80*	*14.30*	*15.40*	*16.03*	*1.08*
10	21.53	21.48	20.66	20.93	20.18	20.44	20.87	0.55
11	23.60	22.80	20.20	22.00	22.00	22.80	22.23	1.16
13	21.81	21.73	21.64	21.92	20.99	21.16	21.54	0.38
14	22.74	21.14	20.11	20.96	19.72	19.11	20.63	1.28

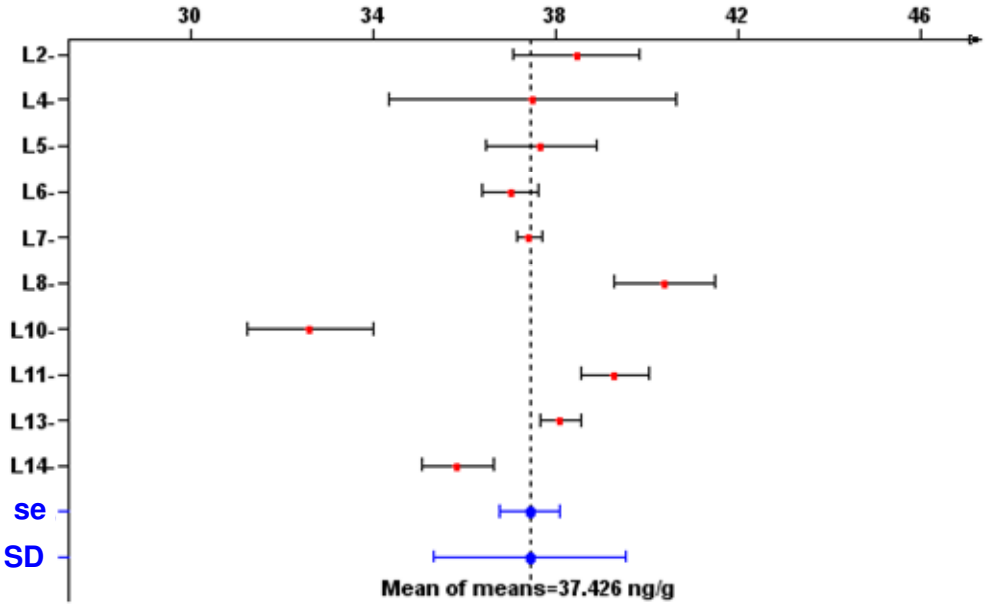
Laboratory means and their standard deviation for PCB 28



PCB 52 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
1*	*23.70*	*20.60*	*26.80*	*21.30*	*25.60*	*25.20*	*23.87*	*2.48*
2	39.70	39.70	36.50	39.50	37.30	38.00	38.45	1.38
*3**	*44.40*	*49.50*	*48.80*	*48.80*	*46.50*	*49.80*	*47.97*	*2.10*
4	35.00	41.00	35.00	42.00	36.00	36.00	37.50	3.15
5	40.00	38.00	37.00	37.00	37.00	37.00	37.67	1.21
6	37.00	37.00	37.00	36.00	38.00	37.00	37.00	0.63
7	37.14	37.57	37.24	37.89	37.21	37.46	37.42	0.28
8	40.36	40.77	42.33	40.09	39.49	39.28	40.39	1.10
9*	*30.80*	*31.40*	*37.00*	*30.50*	*38.40*	*37.00*	*34.18*	*3.64*
10	33.66	30.85	34.32	33.00	31.16	32.70	32.62	1.37
11	40.10	40.10	39.10	38.50	38.40	39.50	39.28	0.75
13	38.47	38.35	38.48	37.91	37.29	38.11	38.10	0.46
14	36.55	34.94	36.25	34.73	36.44	36.13	35.84	0.79

Laboratory means and their standard deviation for PCB 52

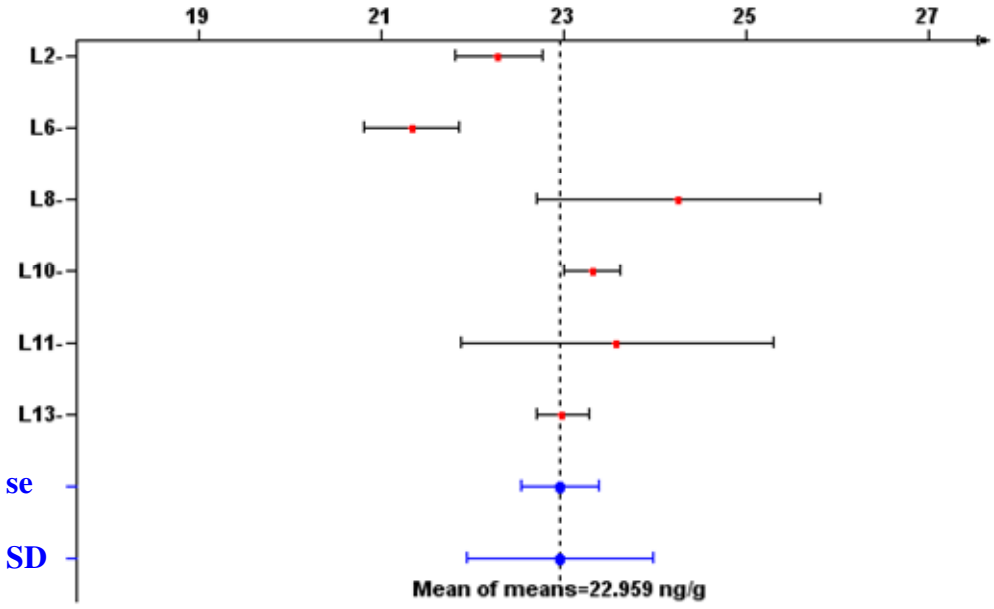


Note: The result of Laboratory # 10 is a statistically significant outlier according to the Nalimov *t*-test (Annex G). However, in combination with the associated measurement uncertainty reported by laboratory # 10 (i.e. 16 %; which is similar to the reported uncertainties of the other laboratories), the measured value is not significantly different from the certified value. The measurement uncertainty is not taken into account by the Nalimov *t*-test.

PCB 74 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
*1**	*24.60*	*21.20*	*24.20*	*22.20*	*23.80*	*24.40*	*23.40*	*1.38*
2	22.20	22.40	21.40	22.60	22.80	22.30	22.28	0.48
6	22.00	21.00	21.00	21.00	22.00	21.00	21.33	0.52
*7**	*37.66*	*38.20*	*38.01*	*35.88*	*41.37*	*37.39*	*38.09*	*1.81*
8	25.54	26.06	24.20	24.85	22.81	22.08	24.26	1.55
*9**	*23.80*	*24.00*	*27.40*	*24.20*	*28.70*	*27.40*	*25.92*	*2.16*
10	23.51	23.56	23.62	23.09	22.85	23.23	23.31	0.31
11	24.00	21.90	24.10	22.00	23.00	26.50	23.58	1.71
13	23.16	22.87	23.48	22.66	22.79	22.96	22.99	0.30

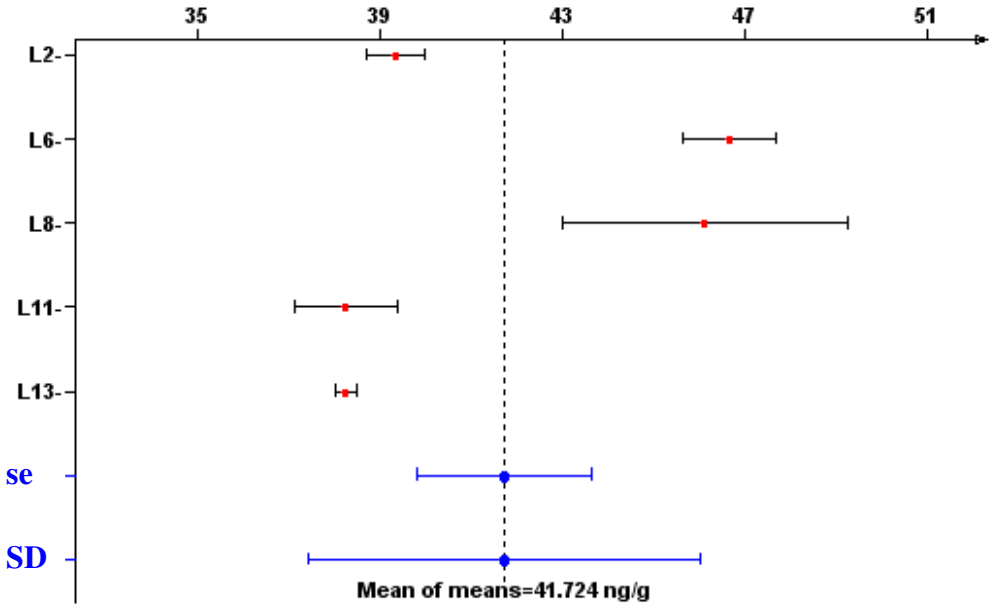
Laboratory means and their standard deviation for PCB 74



PCB 95 mass fraction in ERM-BB350 [ng/g] – NOT CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
2	39.90	39.50	38.40	40.00	39.40	38.80	39.33	0.63
6	48.00	47.00	46.00	47.00	47.00	45.00	46.67	1.03
8	48.90	49.45	44.83	47.77	41.39	44.41	46.13	3.11
9*	*61.60*	*62.70*	*66.70*	*62.90*	*71.00*	*62.30*	*64.53*	*3.64*
11	38.80	36.80	38.20	37.10	38.80	39.80	38.25	1.13
13	38.34	38.49	38.19	38.51	37.98	37.96	38.25	0.24

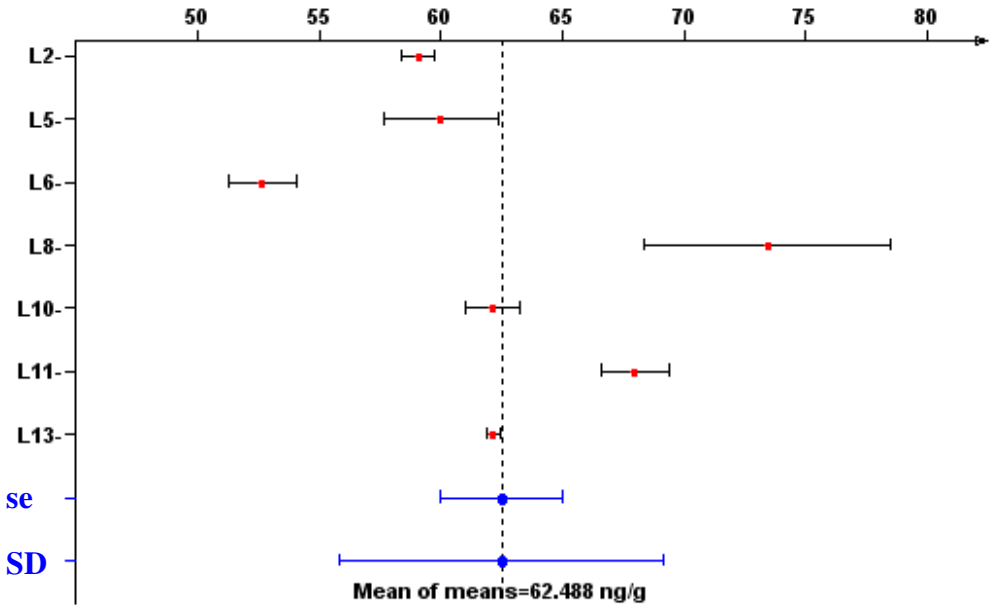
Laboratory means and their standard deviation for PCB 95



PCB 99 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
*1**	*59.90*	*65.00*	*66.80*	*56.10*	*67.40*	*63.60*	*63.13*	*4.37*
2	59.90	59.10	58.20	59.60	59.30	58.30	59.07	0.69
5	59.00	57.00	61.00	58.00	63.00	62.00	60.00	2.37
6	55.00	53.00	52.00	53.00	52.00	51.00	52.67	1.37
*7**	*61.99*	*64.24*	*64.12*	*70.50*	*64.73*	*67.87*	*65.58*	*3.07*
8	78.24	78.58	70.30	76.85	66.58	70.07	73.44	5.09
*9**	*61.60*	*62.70*	*66.70*	*62.90*	*71.00*	*62.30*	*64.53*	*3.64*
10	63.47	62.52	63.15	61.24	60.58	61.77	62.12	1.13
11	68.20	66.30	67.40	66.80	69.40	69.70	67.97	1.38
13	62.04	62.07	62.39	61.76	62.28	62.42	62.16	0.25

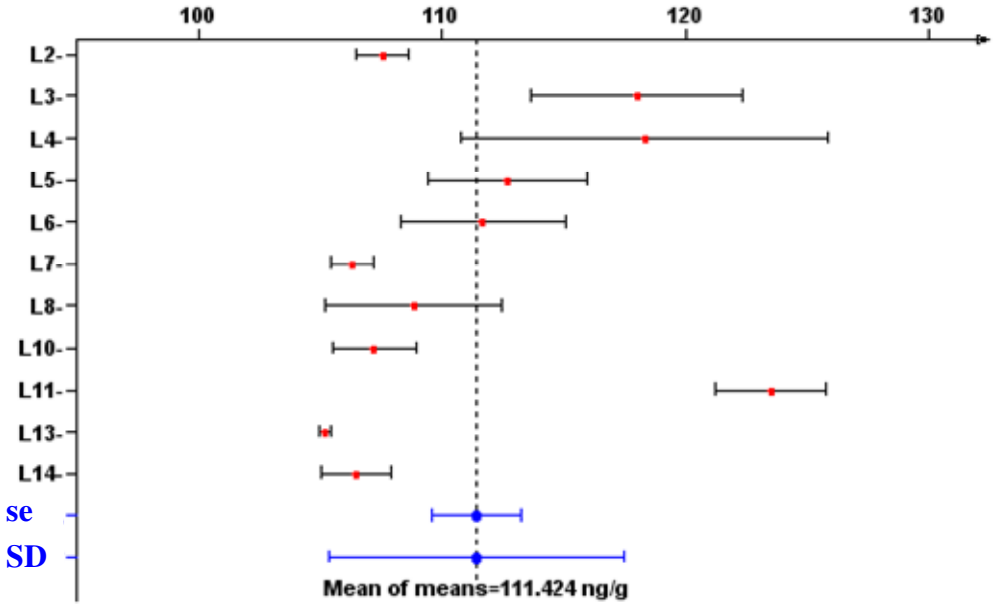
Laboratory means and their standard deviation for PCB 99



PCB 101 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
*1**	*88.00*	*88.60*	*89.50*	*90.90*	*100.60*	*90.30*	*91.32*	*4.67*
2	108.20	107.60	105.90	108.70	108.30	106.60	107.55	1.09
3	113.00	124.00	116.00	121.00	114.00	120.00	118.00	4.34
4	107.00	124.00	113.00	128.00	119.00	119.00	118.33	7.53
5	115.00	107.00	114.00	111.00	116.00	113.00	112.67	3.27
6	118.00	112.00	110.00	112.00	109.00	109.00	111.67	3.39
7	104.61	106.12	106.88	106.43	106.77	106.91	106.29	0.88
8	109.21	114.28	106.38	111.82	104.85	106.41	108.83	3.65
*9**	*87.20*	*87.00*	*104.00*	*85.00*	*104.00*	*94.80*	*93.67*	*8.67*
10	109.58	107.05	108.43	106.58	104.61	107.00	107.21	1.69
11	125.00	124.00	122.00	121.00	122.00	127.00	123.50	2.26
13	105.26	105.26	104.97	104.86	105.51	105.03	105.15	0.24
14	106.63	105.38	108.27	105.13	108.13	105.36	106.48	1.43

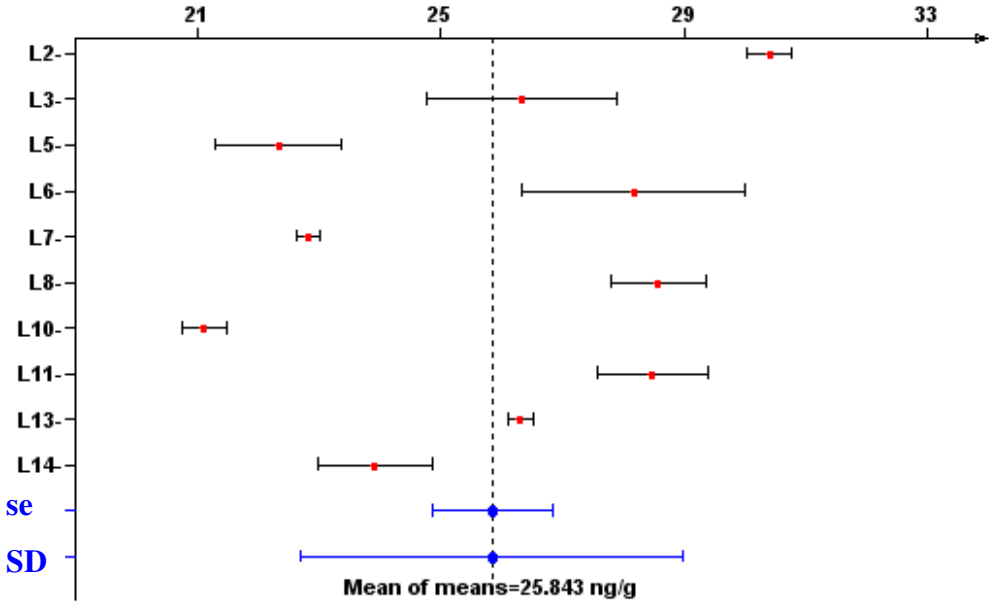
Laboratory means and their standard deviation for PCB 101



PCB 105 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
1*	*20.20*	*19.90*	*22.40*	*20.70*	*24.00*	*22.50*	*21.62*	*1.60*
2	30.40	30.70	30.20	30.30	30.90	29.90	30.40	0.36
3	24.20	27.70	25.70	28.20	25.10	27.00	26.32	1.57
5	22.00	22.00	22.00	21.00	23.00	24.00	22.33	1.03
6	31.00	29.00	27.00	29.00	26.00	27.00	28.17	1.83
7	22.64	22.87	23.10	22.89	22.87	22.57	22.82	0.19
8	28.77	29.89	28.25	28.89	27.73	27.93	28.58	0.79
9*	*20.80*	*20.10*	*19.90*	*20.70*	*20.40*	*20.70*	*20.43*	*0.37*
10	21.44	21.00	21.63	20.97	20.66	20.92	21.10	0.36
11	30.00	28.20	27.70	29.00	27.50	28.50	28.48	0.92
13	26.55	26.42	26.31	26.36	26.24	25.96	26.31	0.20
14	24.23	25.23	22.90	24.54	22.88	23.74	23.92	0.93

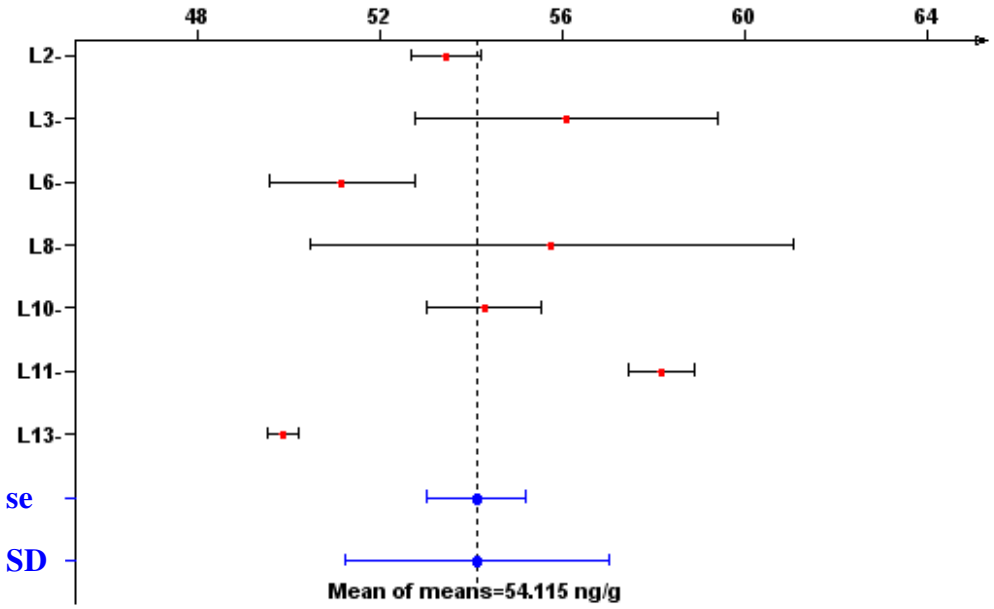
Laboratory means and their standard deviation for PCB 105



PCB 110 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
*1**	*102.10*	*85.70*	*107.50*	*94.10*	*101.00*	*93.60*	*97.33*	*7.74*
2	54.30	53.90	52.60	53.90	53.60	52.40	53.45	0.77
3	51.90	60.10	54.30	59.70	53.90	56.70	56.10	3.32
6	53.00	53.00	51.00	51.00	50.00	49.00	51.17	1.60
*7**	*45.79*	*47.42*	*41.50*	*48.55*	*44.83*	*47.83*	*45.99*	*2.59*
8	59.55	61.08	51.57	60.82	49.19	52.34	55.76	5.30
*9**	*57.70*	*54.20*	*51.50*	*57.40*	*50.00*	*52.10*	*53.82*	*3.19*
10	56.06	54.69	54.68	53.82	52.28	54.15	54.28	1.24
11	57.50	58.80	57.40	58.30	59.20	57.90	58.18	0.72
13	50.12	49.57	50.08	49.32	49.99	50.11	49.87	0.34

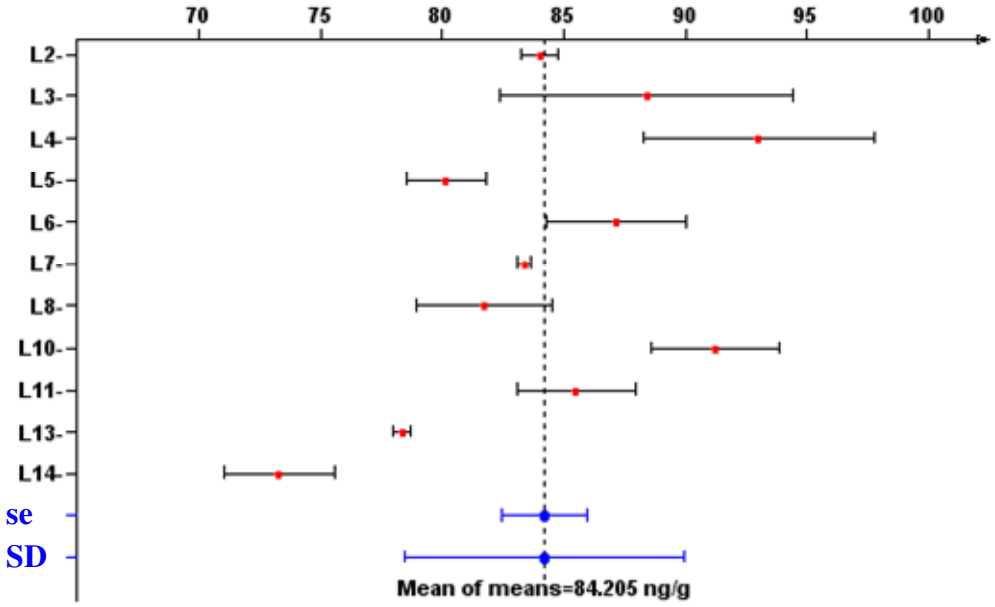
Laboratory means and their standard deviation for PCB 110



PCB 118 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
*1**	*62.10*	*60.90*	*70.20*	*64.30*	*67.60*	*65.80*	*65.15*	*3.46*
2	84.40	83.30	83.60	83.80	85.40	83.60	84.02	0.77
3	77.30	90.50	88.20	89.00	89.90	95.50	88.40	6.01
4	85.00	95.00	91.00	99.00	93.00	95.00	93.00	4.73
5	82.00	79.00	78.00	80.00	82.00	80.00	80.17	1.60
6	91.00	89.00	85.00	89.00	85.00	84.00	87.17	2.86
7	83.13	83.88	83.23	83.47	83.50	83.16	83.40	0.28
8	82.22	84.61	80.89	84.66	77.26	80.70	81.72	2.79
*9**	*71.50*	*68.70*	*62.60*	*69.60*	*61.50*	*62.90*	*66.13*	*4.29*
10	95.58	91.32	92.70	89.54	88.47	89.72	91.22	2.60
11	87.70	87.80	85.50	81.80	83.50	86.70	85.50	2.42
13	78.70	78.69	78.23	78.35	78.44	77.72	78.36	0.36
14	73.78	75.33	72.66	69.77	72.28	76.02	73.31	2.27

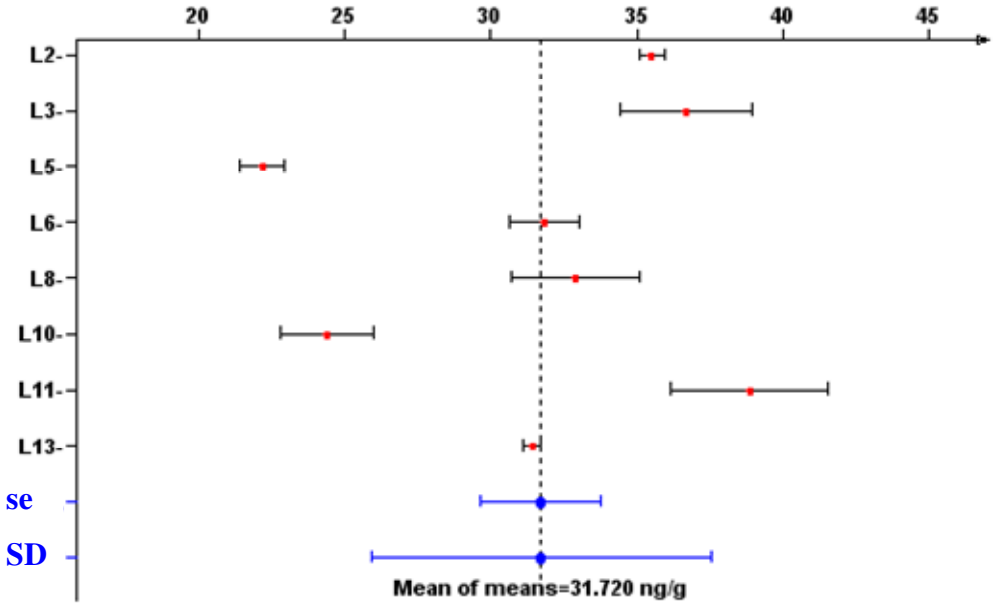
Laboratory means and their standard deviation for PCB 118



PCB 128 mass fraction in ERM-BB350 [ng/g] – NOT CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
1*	*19.80*	*23.80*	*21.90*	*24.80*	*24.30*	*23.40*	*23.00*	*1.85*
2	35.20	35.50	34.80	35.80	36.00	35.80	35.52	0.45
3	32.40	37.00	36.80	37.80	37.00	39.10	36.68	2.26
5	22.00	22.00	23.00	21.00	23.00	22.00	22.17	0.75
6	34.00	32.00	31.00	32.00	31.00	31.00	31.83	1.17
7*	*25.75*	*26.44*	*26.92*	*26.61*	*26.33*	*27.28*	*26.56*	*0.52*
8	31.07	32.29	33.50	30.08	34.14	36.16	32.87	2.20
9*	*31.40*	*30.80*	*28.70*	*34.80*	*27.60*	*28.10*	*30.23*	*2.70*
10	25.02	26.13	22.80	26.28	23.31	22.94	24.41	1.60
11	43.60	37.60	40.00	36.00	38.70	37.30	38.87	2.68
13	31.70	31.68	31.55	31.40	31.07	31.06	31.41	0.29

Laboratory means and their standard deviation for PCB 128

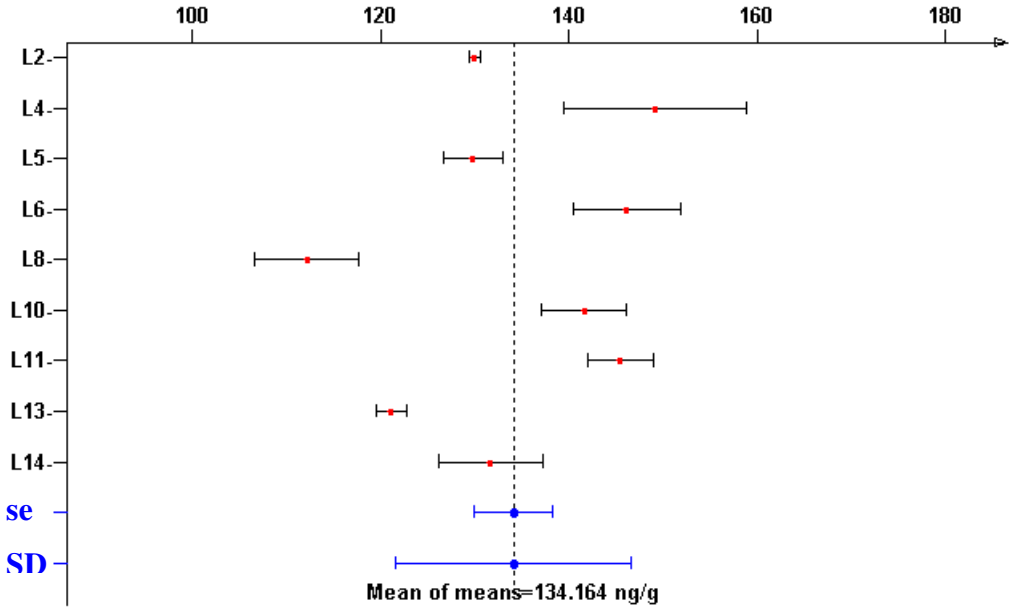


The results of laboratories # 5 and # 10 are not in agreement with the consensus value following a trueness test according to IRMM Application Note 1 [20]. It was therefore decided not to certify the mass fraction for this congener.

PCB 138 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
1*	*123.10*	*133.70*	*146.00*	*140.10*	*150.90*	*154.00*	*141.30*	*11.54*
2	129.70	130.90	130.00	129.60	130.70	129.30	130.03	0.64
3*	*147.00*	*170.00*	*157.00*	*171.00*	*159.00*	*168.00*	*162.00*	*9.38*
4	134.00	150.00	143.00	161.00	150.00	157.00	149.17	9.70
5	132.00	129.00	134.00	128.00	131.00	125.00	129.83	3.19
6	153.00	151.00	140.00	148.00	139.00	146.00	146.17	5.71
7*	*198.92*	*199.60*	*200.12*	*201.63*	*199.64*	*200.66*	*200.10*	*0.95*
8	107.08	109.36	115.95	107.54	112.12	121.30	112.23	5.53
9*	*96.60*	*99.80*	*81.40*	*98.00*	*84.50*	*77.70*	*89.67*	*9.58*
10	148.56	145.33	140.77	140.97	136.87	137.39	141.65	4.55
11	150.00	144.00	145.00	148.00	140.00	146.00	145.50	3.45
13	122.32	123.15	120.81	121.89	119.60	119.28	121.18	1.55
14	132.56	124.51	135.79	125.59	137.98	133.95	131.73	5.50

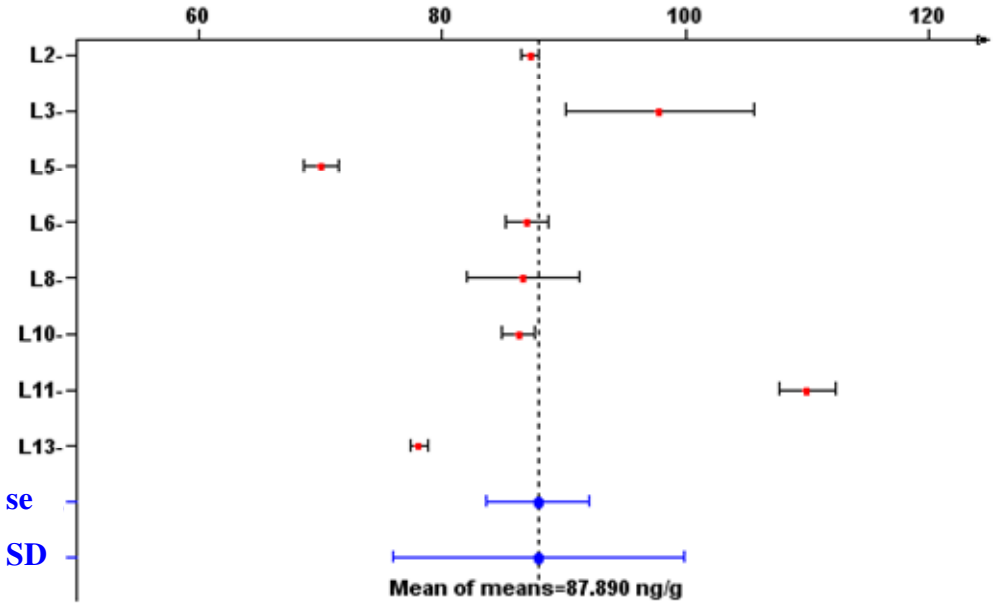
Laboratory means and their standard deviation for PCB 138



PCB 149 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
*1**	*51.10*	*45.90*	*58.70*	*47.60*	*57.00*	*51.20*	*51.92*	*5.06*
2	87.90	87.80	86.40	87.20	87.80	86.30	87.23	0.73
3	84.50	96.80	100.00	96.90	101.00	108.00	97.87	7.72
5	70.00	70.00	72.00	68.00	71.00	69.00	70.00	1.41
6	90.00	88.00	87.00	86.00	86.00	85.00	87.00	1.79
*7**	*83.84*	*84.63*	*89.68*	*195.36*	*81.88*	*202.76*	*123.03*	*59.00*
8	81.45	86.34	89.73	80.67	90.72	91.04	86.66	4.65
*9**	*75.20*	*75.00*	*57.50*	*77.00*	*57.20*	*57.00*	*66.48*	*10.16*
10	87.57	88.02	84.75	86.39	85.01	85.76	86.25	1.34
11	106.00	109.00	112.00	112.00	111.00	110.00	110.00	2.28
13	77.49	77.84	78.65	77.16	78.44	79.07	78.11	0.73

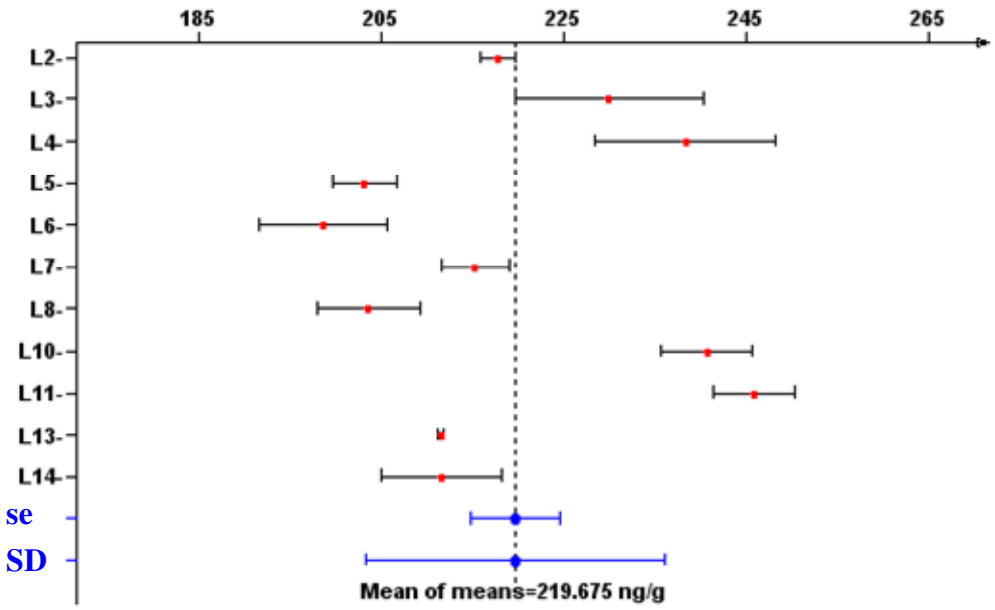
Laboratory means and their standard deviation for PCB 149



PCB 153 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
1*	*187.00*	*185.00*	*198.50*	*195.40*	*196.80*	*192.80*	*192.58*	*5.47*
2	219.50	218.50	215.90	217.40	219.80	215.50	217.77	1.81
3	213.00	236.00	226.00	236.00	227.00	242.00	230.00	10.30
4	222.00	245.00	234.00	251.00	238.00	240.00	238.33	9.93
5	208.00	202.00	203.00	198.00	206.00	202.00	203.17	3.49
6	209.00	203.00	197.00	199.00	188.00	196.00	198.67	7.06
7	212.12	215.45	211.32	219.70	213.54	219.63	215.29	3.67
8	203.37	202.93	208.23	194.85	201.19	211.00	203.60	5.64
9*	*181.00*	*182.00*	*147.00*	*183.00*	*154.00*	*147.00*	*165.67*	*18.08*
10	248.82	240.71	242.15	236.93	234.29	240.96	240.64	4.97
11	252.00	240.00	247.00	245.00	242.00	249.00	245.83	4.45
13	211.05	211.79	211.77	211.64	211.16	211.70	211.52	0.33
14	220.52	201.57	215.35	209.39	208.53	214.32	211.61	6.57

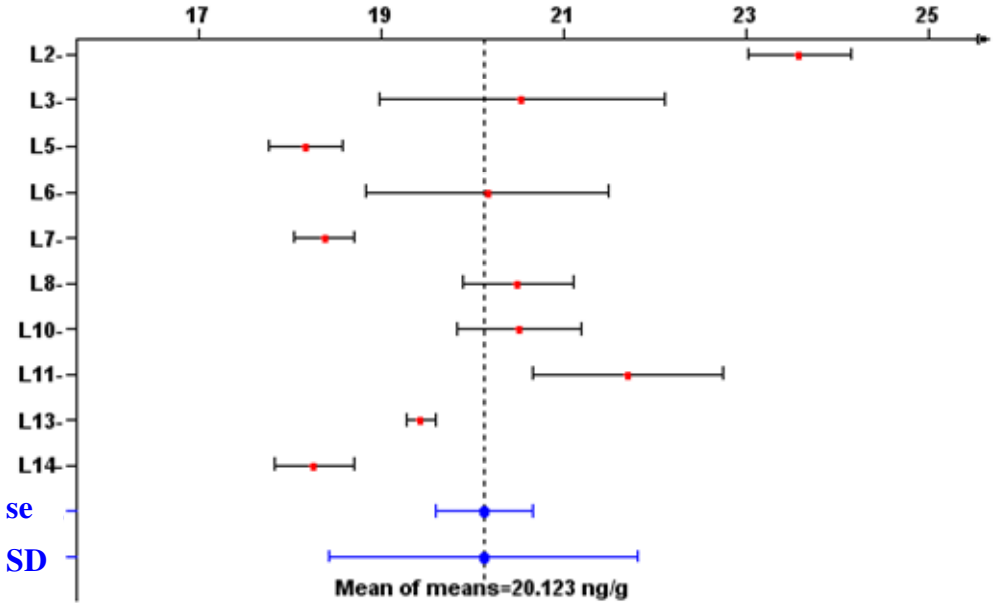
Laboratory means and their standard deviation for PCB 153



PCB 156 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
*1**	*20.80*	*20.00*	*21.60*	*20.90*	*21.90*	*20.90*	*21.02*	*0.67*
2	23.90	23.40	22.60	23.70	24.30	23.60	23.58	0.57
3	17.80	21.60	21.40	21.20	19.50	21.70	20.53	1.56
5	19.00	18.00	18.00	18.00	18.00	18.00	18.17	0.41
6	22.00	21.00	19.00	21.00	19.00	19.00	20.17	1.33
7	18.11	18.51	18.92	18.47	18.04	18.20	18.38	0.33
8	20.11	20.61	20.85	19.49	21.23	20.68	20.50	0.61
*9**	*14.90*	*15.20*	*13.90*	*15.30*	*14.40*	*13.60*	*14.55*	*0.70*
10	21.68	20.57	20.68	20.32	19.63	20.19	20.51	0.68
11	23.20	21.20	20.20	21.30	21.90	22.40	21.70	1.04
13	19.51	19.46	19.24	19.70	19.33	19.35	19.43	0.16
14	18.28	18.03	18.08	18.08	17.99	19.12	18.26	0.43

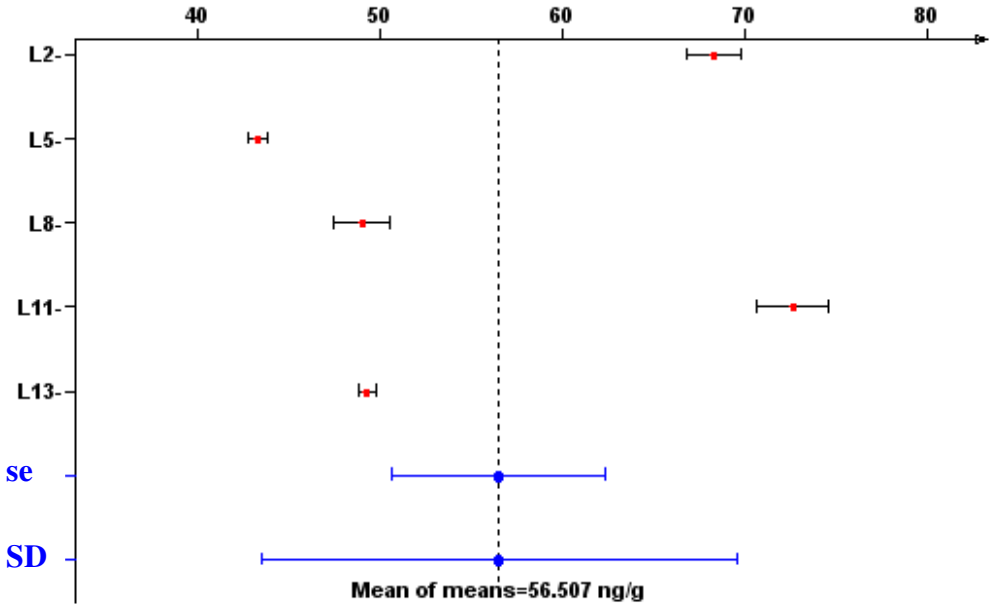
Laboratory means and their standard deviation for PCB 156



PCB 163 mass fraction in ERM-BB350 [ng/g] – NOT CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
2	67.10	69.80	67.70	66.30	69.00	69.90	68.30	1.49
5	43.00	43.00	44.00	43.00	43.00	44.00	43.33	0.52
8	48.85	49.44	48.85	48.94	46.60	51.29	49.00	1.50
9*	*49.00*	*48.80*	*37.00*	*48.40*	*37.80*	*36.50*	*42.92*	*6.39*
11	71.20	75.00	74.00	72.40	69.60	73.50	72.62	1.98
13	49.44	49.15	49.79	49.32	49.58	48.46	49.29	0.46

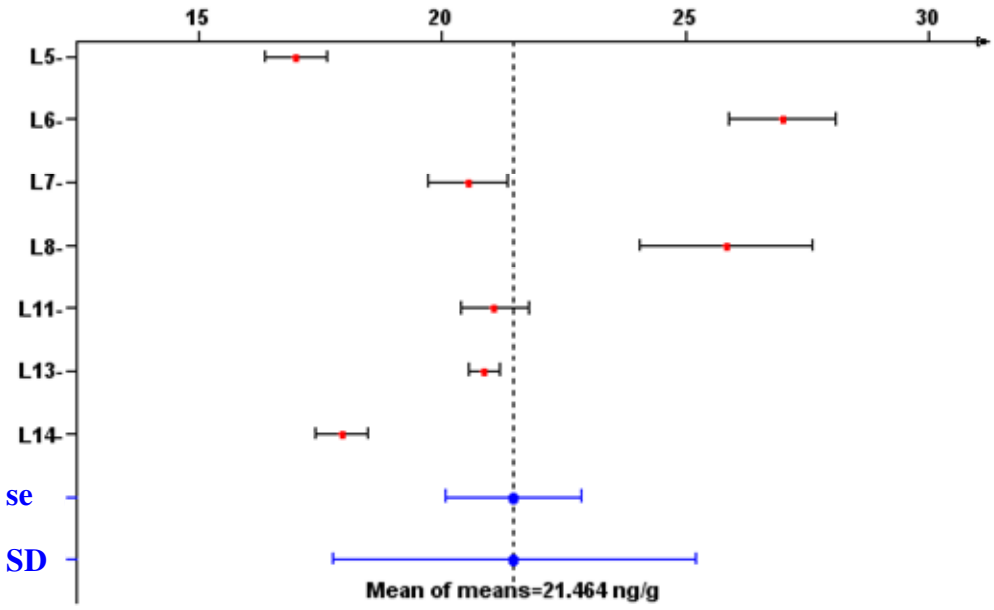
Laboratory means and their standard deviation for PCB 163



PCB 167 mass fraction in ERM-BB350 [ng/g]- NOT CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
*1**	*20.50*	*18.30*	*24.70*	*21.10*	*23.20*	*22.80*	*21.77*	*2.27*
*2**	*30.90*	*31.60*	*30.80*	*30.50*	*30.70*	*30.40*	*30.82*	*0.43*
5	17.00	17.00	17.00	16.00	17.00	18.00	17.00	0.63
6	28.00	28.00	26.00	28.00	26.00	26.00	27.00	1.10
7	20.68	19.77	21.46	19.32	21.16	20.81	20.53	0.83
8	27.89	27.57	23.92	26.83	24.32	24.49	25.84	1.79
*9**	*16.20*	*16.00*	*14.60*	*15.40*	*15.30*	*14.70*	*15.37*	*0.65*
11	21.90	21.80	20.00	21.10	20.70	21.00	21.08	0.71
13	21.40	20.45	20.94	20.73	20.85	20.71	20.85	0.32
14	17.67	17.79	17.84	17.58	17.77	19.02	17.95	0.53

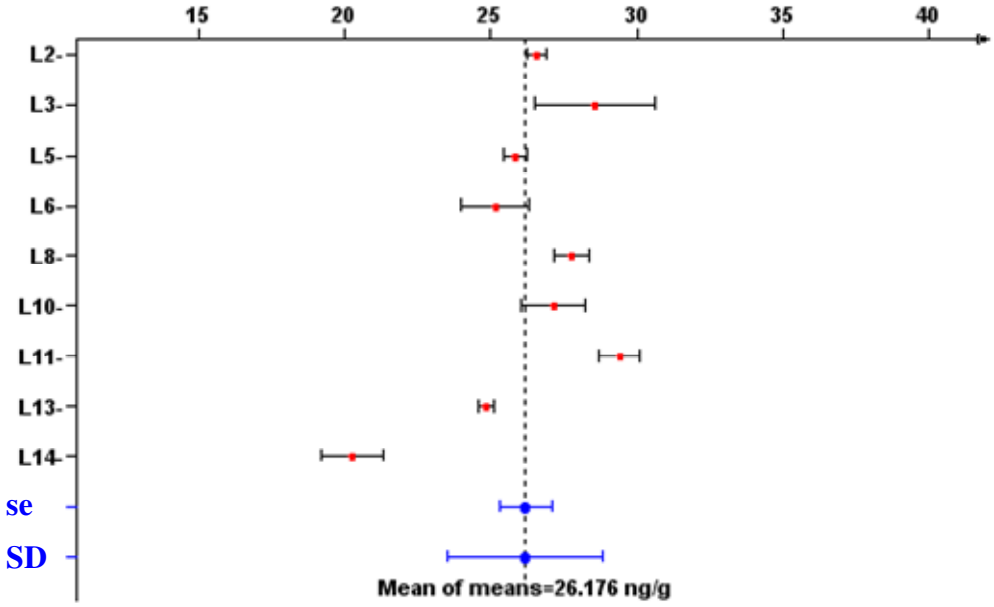
Laboratory means and their standard deviation for PCB 167



PCB 170 mass fraction in ERM-BB350 [ng/g] – NOT CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
1*	*24.90*	*24.00*	*29.30*	*25.90*	*29.40*	*28.20*	*26.95*	*2.33*
2	26.30	26.70	26.40	26.80	27.00	26.20	26.57	0.31
3	25.30	30.00	28.90	28.80	27.30	31.20	28.58	2.07
5	26.00	26.00	25.00	26.00	26.00	26.00	25.83	0.41
6	27.00	26.00	25.00	25.00	24.00	24.00	25.17	1.17
7*	*29.77*	*34.44*	*26.57*	*48.15*	*30.53*	*40.70*	*35.03*	*8.05*
8	27.84	28.70	28.19	27.40	27.05	27.44	27.77	0.60
9*	*19.00*	*18.80*	*17.20*	*18.50*	*17.80*	*18.20*	*18.25*	*0.67*
10	29.15	27.10	27.00	27.38	26.03	26.27	27.15	1.10
11	29.90	29.90	28.70	29.20	28.50	30.20	29.40	0.70
13	24.93	25.00	24.90	25.03	24.86	24.31	24.84	0.27
14	19.20	20.29	20.49	18.91	20.91	21.80	20.27	1.08

Laboratory means and their standard deviation for PCB 170

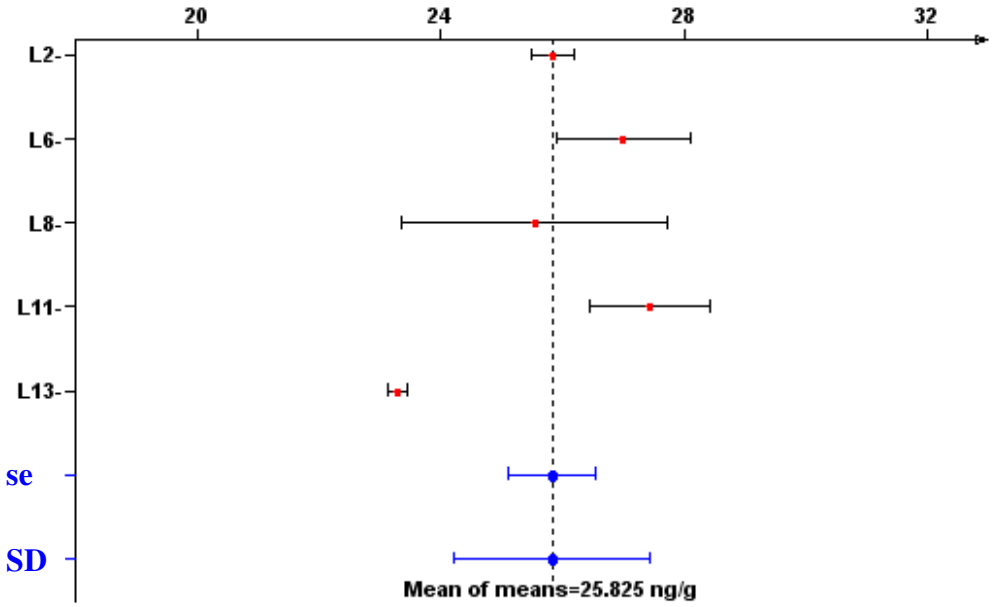


Note: The result of Laboratory # 14 is a statistically significant outlier according to the Nalimov *t*-test (Annex G). No technical reasons were identified to exclude this value. The value of this congener is therefore not certified.

PCB 177 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
2	26.10	25.90	25.70	25.90	26.20	25.20	25.83	0.36
6	29.00	27.00	27.00	27.00	26.00	26.00	27.00	1.10
9*	*20.40*	*19.70*	*19.10*	*21.50*	*17.40*	*19.30*	*19.57*	*1.37*
8	27.50	27.58	23.32	27.51	23.84	23.52	25.55	2.18
11	26.40	27.00	27.10	27.60	27.30	29.30	27.45	0.99
13	23.30	23.44	23.48	23.10	23.34	23.11	23.29	0.16

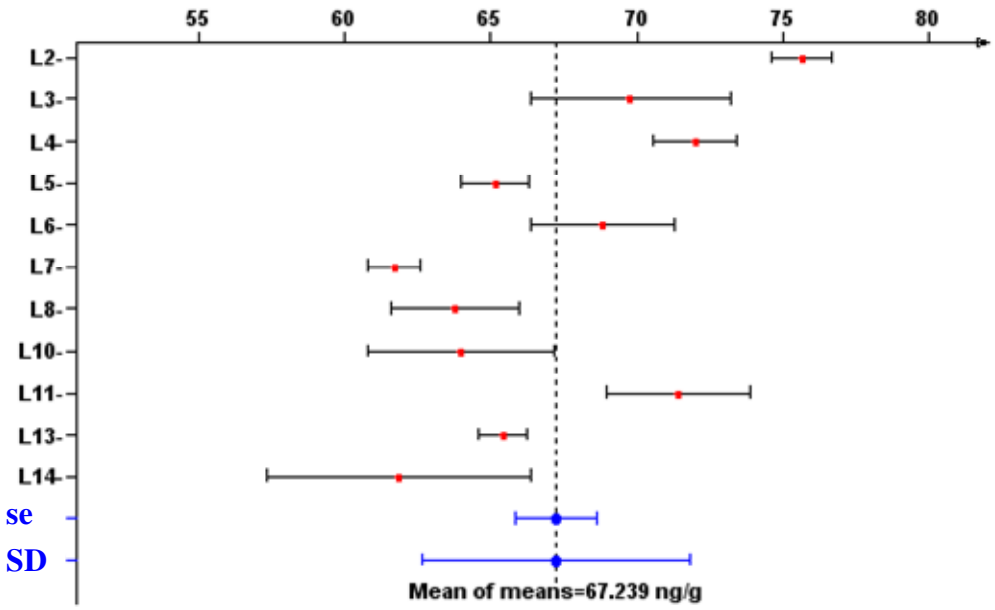
Laboratory means and their standard deviation for PCB 177



PCB 180 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
*1**	*66.20*	*64.10*	*73.00*	*65.60*	*72.20*	*71.90*	*68.83*	*3.95*
2	75.30	75.10	76.50	74.30	77.10	75.60	75.65	1.01
3	64.70	70.60	72.40	71.10	66.50	73.40	69.78	3.43
4	70.00	71.00	72.00	72.00	73.00	74.00	72.00	1.41
5	67.00	65.00	64.00	66.00	65.00	64.00	65.17	1.17
6	72.00	70.00	67.00	71.00	66.00	67.00	68.83	2.48
7	61.14	61.89	61.54	63.23	60.63	61.73	61.69	0.88
8	66.66	66.54	61.95	62.19	62.50	62.97	63.80	2.19
*9**	*60.00*	*59.70*	*55.50*	*58.00*	*57.60*	*57.90*	*58.12*	*1.63*
10	66.95	67.60	61.87	65.80	60.12	61.56	63.98	3.18
11	75.70	72.20	70.20	71.40	70.80	68.30	71.43	2.47
13	65.70	65.91	65.64	64.13	66.43	64.67	65.41	0.85
14	65.61	65.88	59.92	66.00	55.94	57.89	61.87	4.52

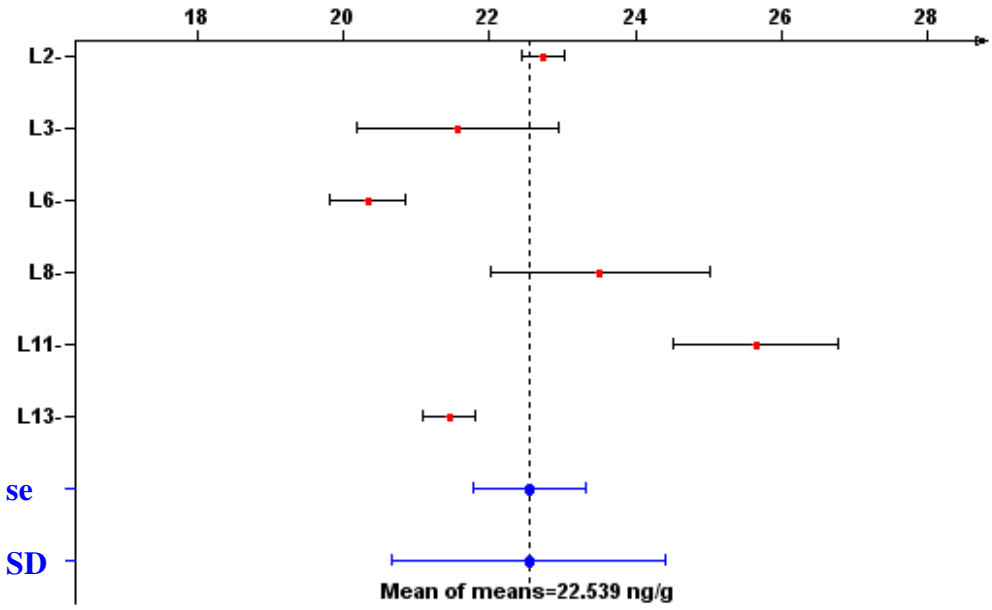
Laboratory means and their standard deviation for PCB 180



PCB 183 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
*1**	*18.00*	*17.10*	*19.50*	*18.00*	*19.20*	*18.90*	*18.45*	*0.90*
2	22.80	22.70	23.00	22.20	22.90	22.80	22.73	0.28
3	19.80	23.10	20.90	23.30	20.80	21.50	21.57	1.38
6	21.00	21.00	20.00	20.00	20.00	20.00	20.33	0.52
*7**	*26.10*	*26.12*	*25.68*	*15.22*	*23.17*	*11.07*	*21.23*	*6.49*
8	25.12	25.27	22.12	24.09	22.22	22.22	23.51	1.50
*9**	*22.00*	*21.50*	*18.10*	*23.10*	*15.70*	*17.40*	*19.63*	*2.96*
11	25.40	25.20	24.60	25.90	25.00	27.80	25.65	1.14
13	21.27	21.31	21.79	20.88	21.82	21.61	21.44	0.36

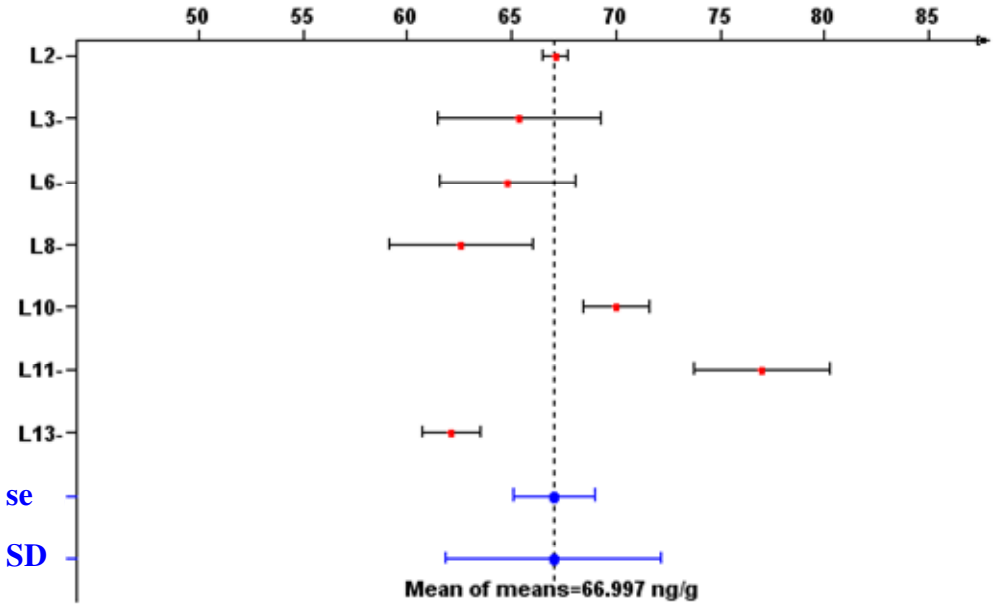
Laboratory means and their standard deviation for PCB 183



PCB 187 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
*1**	*35.80*	*35.00*	*36.90*	*37.00*	*35.90*	*36.00*	*36.10*	*0.75*
2	67.00	67.40	67.20	66.40	68.10	66.50	67.10	0.63
3	59.00	67.10	65.80	68.70	62.60	69.10	65.38	3.91
6	70.00	67.00	63.00	65.00	61.00	63.00	64.83	3.25
*7**	*72.66*	*74.13*	*66.41*	*61.10*	*65.15*	*46.65*	*64.35*	*9.93*
8	66.73	66.54	60.04	63.15	59.09	59.80	62.56	3.45
*9**	*56.40*	*56.80*	*50.60*	*59.90*	*46.30*	*52.40*	*53.73*	*4.93*
10	72.80	70.42	70.33	69.44	68.45	68.68	70.02	1.58
11	80.00	75.00	73.40	75.70	75.90	81.90	76.98	3.25
13	61.50	61.93	63.09	59.69	63.53	62.87	62.10	1.40

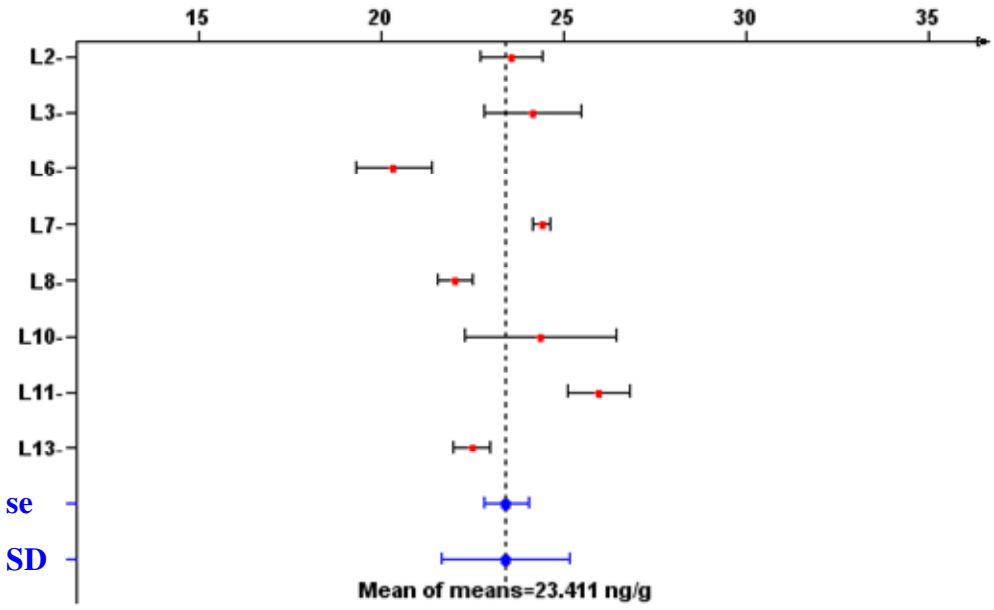
Laboratory means and their standard deviation for PCB 187



PCB 194 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
*1**	*23.50*	*22.80*	*26.00*	*23.90*	*25.50*	*24.80*	*24.42*	*1.23*
2	22.90	23.80	24.50	22.30	24.40	23.60	23.58	0.86
3	23.60	26.00	23.00	25.30	22.60	24.50	24.17	1.33
6	22.00	21.00	20.00	20.00	19.00	20.00	20.33	1.03
7	24.40	24.59	24.18	24.70	24.11	24.41	24.40	0.23
8	22.42	22.42	21.83	22.38	21.89	21.16	22.02	0.50
*9**	*23.10*	*22.90*	*15.30*	*17.30*	*14.30*	*15.10*	*18.00*	*4.00*
10	27.03	26.29	22.83	25.15	22.34	22.54	24.36	2.06
11	27.10	26.10	24.70	26.60	25.30	25.90	25.95	0.87
13	21.95	22.02	22.78	22.22	22.65	23.26	22.48	0.51

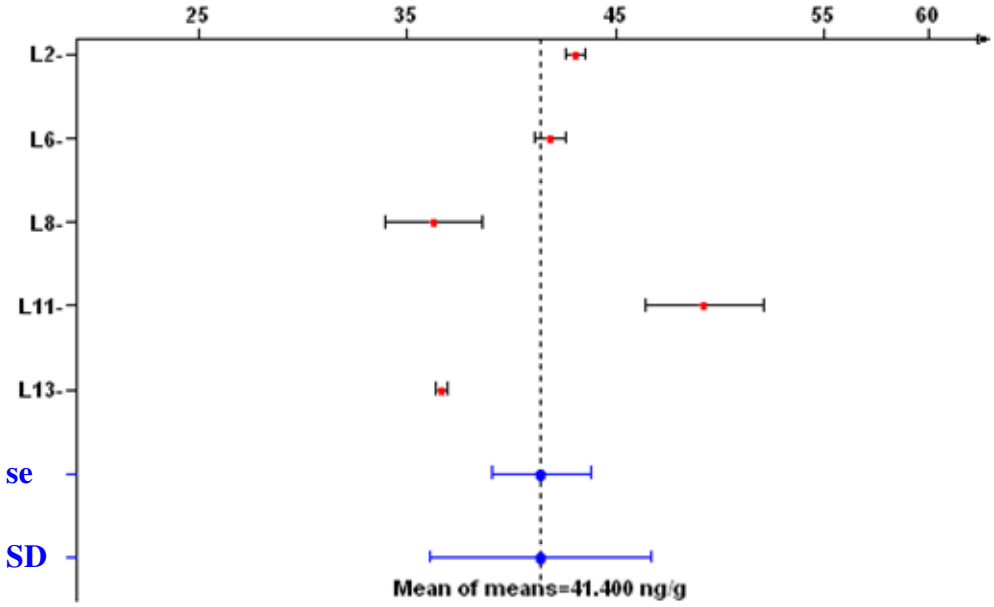
Laboratory means and their standard deviation for PCB 194



PCB 196 mass fraction in ERM-BB350 [ng/g] - CERTIFIED

Lab code	Day 1/1	Day 1/2	Day 1/3	Day 2/1	Day 2/2	Day 2/3	Mean	SD
*1**	*27.10*	*26.40*	*28.50*	*27.90*	*28.60*	*28.00*	*27.75*	*0.85*
2	43.20	43.40	42.30	42.60	43.50	43.30	43.05	0.48
6	43.00	42.00	42.00	41.00	41.00	42.00	41.83	0.75
8	38.35	38.80	33.83	37.60	33.56	35.38	36.25	2.31
*9**	*37.30*	*39.90*	*29.40*	*36.30*	*27.00*	*29.00*	*33.15*	*5.33*
11	50.10	52.80	46.70	52.20	46.30	47.30	49.23	2.86
13	36.58	36.36	37.20	36.43	36.74	36.46	36.63	0.31

Laboratory means and their standard deviation for PCB 196



European Commission

EUR 24424 EN – Joint Research Centre – Institute for Reference Materials and Measurements
Title: Certification of mass fractions of polychlorinated biphenyls (PCBs 28, 52, 74, 99, 101, 105, 110, 118, 138, 149, 153, 156, 177, 180, 183, 187, 194 and 196) in fish oil
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Abstract
This report describes the preparation of a salmon oil matrix certified reference material (ERM-BB350) and the certification of the content (mass fraction) of a selection of polychlorinated biphenyls (Nos. 28, 52, 74, 99, 101, 105, 110, 118, 138, 149, 153, 156, 177, 180, 183, 187, 194 and 196).
Certification of the CRM included testing of the homogeneity and stability of the material as well as the characterisation using an inter-comparison approach. The main purpose of the material is to assess method performance, i.e. for checking accuracy of analytical results. As any reference material, the CRM can also be used for control charts or validation studies.
Uncertainties were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [1] and include uncertainties due to possible heterogeneity, instability and characterisation. The certified values are listed below:

FISH OIL

IUPAC name (congener number) [1)]	Mass Fraction	
	Certified value [2)] [ng/g]	Uncertainty [3)] [ng/g]
2,4,4'-trichlorobiphenyl (PCB 28)	21.3	1.1
2,2',5,5'-tetrachlorobiphenyl (PCB 52)	37.4	2.2
2,4,4',5-tetrachlorobiphenyl (PCB 74)	23.0	1.9
2,2',4,4',5-pentachlorobiphenyl (PCB 99)	62	6
2,2',4,5,5'-pentachlorobiphenyl (PCB 101)	111	5
2,3,3',4,4'-pentachlorobiphenyl (PCB 105	25.8	2.1
2,3,3',4',6-pentachlorobiphenyl (PCB 110)	54.1	2.8
2,3',4,4',5-pentachlorobiphenyl (PCB 118)	84	4
2,2',3,4,4',5'-hexachlorobiphenyl (PCB 138)	137	10
2,2',3,4',5',6-hexachlorobiphenyl (PCB 149)	88	9
2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153)	220	11
2,3,3',4,4',5-hexachlorobiphenyl (PCB 156)	20.1	1.3
2,2',3,3',4,5',6'-heptachlorobiphenyl (PCB 177)	25.8	2.0
2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB 180)	67	4
2,2',3,4,4',5',6-heptachlorobiphenyl (PCB 183)	22.5	1.8
2,2',3,4',5,5',6-heptachlorobiphenyl (PCB 187)	67	5
2,2',3,3',4,4',5,5'-octachlorobiphenyl (PCB 194)	23.4	1.5
2,2',3,3',4,4',5,6'-octachlorobiphenyl (PCB 196)	41	7

1) As obtained by quantification using gas chromatographic methods. Numbering identical to that published by Ballschmiter K, Bacher R, Mennel A, Fischer R, Riehle U, Swerve M (1992) Journal of high-resolution chromatography 15, 206.

2) Unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory. The certified value and its uncertainty are traceable to the International System of Units (SI).

3) The certified uncertainty is the expanded uncertainty estimated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM) with a coverage factor $k = 2$ (with the exception of PCB 177 and 196, $k = 2.39$ and 2.66, respectively) corresponding to a level of confidence of about 95 %.

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